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# THE PHILIPPINE JOURNAL OF SCIENCE

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## SECTION B TROPICAL MEDICINE

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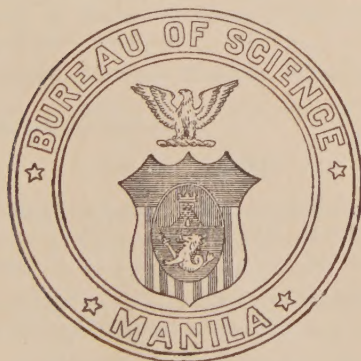
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# THE PHILIPPINE JOURNAL OF SCIENCE

## B. TROPICAL MEDICINE

VOL. XI

JANUARY, 1916

No. 1

### PRESERVATION OF HUMAN SERUM FOR WASSERMANN REACTION<sup>1</sup>

By E. H. RÜDIGER

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Serologists, and laboratory workers in general, whose task it is to perform the Wassermann reaction for syphilis, are frequently annoyed with specimens that are utterly unfit for examination. The physician, many miles from the laboratory, collects the specimen without the necessary aseptic precautions, puts it into an unsterilized bottle, and sends it to the laboratory, where it arrives a week or so later and is nothing but a mass of putrid blood. Many methods for conducting the Wassermann reaction have been proposed, but little or no attention has been paid to the collection and the preparation of the specimens to be examined.

The investigation here to be reported was undertaken with the view of finding a suitable chemical agent that would render the serum sterile and keep it sterile without interfering with the test to be conducted. Of chemical agents, phenol, lysol, tricresol, chloroform, formalin, and glycerin were tried. Phenol, lysol, tricresol, and chloroform were soon abandoned because of the precipitate they produced when added to serum in quantities large enough to destroy microorganisms. Formalin and glycerin were suitable in this respect and were tested to some extent.

*Preparation of the specimen.*—About 10 cubic centimeters of physiologic salt solution were put into each of a number of suitable test tubes. The tubes were stoppered with cotton and were sterilized in the autoclave. The sterilized tubes with the salt solution were stored until needed. Salt solution was put into the tubes to prevent the blood clot from adhering to the wall of the tube, which frequently happens when blood is put into a dry tube. Immediately before use the salt solution was poured out of the test tube, and the blood was put in by letting it flow down the inside of the tube to prevent frothing. The

<sup>1</sup> Received for publication February 17, 1916.

tube with the blood was left at room temperature for two or three hours to allow the blood to clot. If the clot adhered to the tube and did not sink in the serum, it was loosened with a sterile wire and was pushed down into the serum. The specimen was now put into the refrigerator for from twelve to twenty-four hours to allow the serum to separate and the loose corpuscles to settle to the bottom of the tube. After the corpuscles had settled, the clear serum was pipetted off and was mixed with the preservative, after which it was tested at suitable intervals.

*Antigen.*—The antigen used was plain alcoholic extract of human heart-muscle, and about one fourth the anticomplementary dose was used per tube in the test.

*Complement.*—As alien complement the pooled sera of three guinea pigs were used in quantities of 0.1, 0.05, and 0.025 cubic centimeter.

*Hæmolytic amboceptor.*—As artificial hæmolytic amboceptor the antihuman amboceptor advocated by Noguchi was used in doses of 1 to 1.5 unit per tube. The smallest quantity of amboceptor that with 0.05 cubic centimeter of complement dissolved the test dose of corpuscles in one hour was called a unit.

*Corpuscles.*—Human corpuscles were well washed, and 0.5 cubic centimeter of a 4 per cent suspension in physiologic salt solution was used per tube.

*Glassware.*—Two sets of test tubes were used—one set as antigen tubes and another set as control tubes. Test tubes that had once been used as antigen tubes were never used as control tubes. Other glassware, such as pipettes, graduates, beakers, and flasks, that was used for serum was used for serum only, and that which was used for antigen was used for antigen only.

*Methods.*—Two methods of conducting the serum test for syphilis were used—namely, the Wassermann method with human hæmolytic system and the method described by Tschernogubow,<sup>2</sup> Hecht,<sup>3</sup> Gurd,<sup>4</sup> and others. As human serum as a rule dissolves sheep corpuscles better than the corpuscles of the guinea pig, sheep corpuscles were used, as first advocated by Tschernogubow.

*Technique of conducting the Wassermann reaction.*—Unless unheated serum was tested for anticomplementary properties, the serum was heated to between 55° C. and 56° C. for thirty minutes before testing. Six test tubes, three, designated as 1, 2, and 3 antigen tubes, and three, designated as 1', 2', and 3'

<sup>2</sup> *Deut. med. Wochenschr.* (1909), 35, 668.

<sup>3</sup> *Wien. klin. Wochenschr.* (1909), 22, 338.

<sup>4</sup> *Journ. Infect. Dis.* (1911), 8, 427.



control tubes, were used. Of the serum to be tested, 0.6 cubic centimeter was diluted with 2.4 cubic centimeters of physiologic salt solution, and 0.5 cubic centimeter of the diluted serum was put into each of the six test tubes. Each tube of the first pair, tubes 1 and 1', received 0.5 cubic centimeter of 1:5 dilution of complement serum. Of the second pair of tubes, tubes 2 and 2', each received 0.5 cubic centimeter of 1:10 dilution of complement serum, and each of the third pair of tubes, tubes 3 and 3', received 0.5 cubic centimeter of 1:20 dilution of complement serum. Each antigen tube received 0.5 cubic centimeter of diluted antigen representing about one fourth of the anticomplementary dose, and to each of the control tubes 0.5 cubic centimeter of physiologic salt solution was added. Now the tubes were placed in the incubator at 37° C. for one hour. After an hour in the incubator 1 cubic centimeter of sensitized corpuscles was added to each tube; the tubes were well shaken; returned to the incubator for one hour, during which time they were shaken at least three times; were removed to room temperature; and the results were read about three hours after the corpuscles had been added.

*Technique of conducting the Tschernogubow modification of the Wassermann reaction.*—This method utilizes complement and hæmolytic amboceptor normally present in human serum. When fresh serum was tested, the complement and hæmolytic amboceptor were derived from the serum tested, while old serum, heated or unheated, was reactivated with normal human serum.

Ten test tubes, five antigen tubes marked 1, 2, 3, 4, and 5 and five control tubes marked 1', 2', 3', 4', and 5', were used for each test. If the serum to be tested was fresh and unheated, 1.6 cubic centimeters of serum were diluted to 4 cubic centimeters with physiologic salt solution. Each tube of the first pair, tubes 1 and 1', received 1 cubic centimeter of diluted serum representing 0.4 cubic centimeter of serum. The remaining 2 cubic centimeters of diluted serum were further diluted with 2 cubic centimeters of physiologic salt solution, and 1 cubic centimeter of this dilution was put into each of the second pair of tubes, tubes 2 and 2'. Each of the second pair of tubes received 0.2 cubic centimeter of serum. These dilutions were continued until all of the five pairs of tubes were supplied with diluted serum. The quantities of serum represented in the tubes were 0.4, 0.2, 0.1, 0.05, and 0.025 cubic centimeter. Each of the antigen tubes received 1 cubic centimeter of diluted antigen representing about one fourth of the anticomplementary dose, and each control tube received 1 cubic centimeter of physiologic

salt solution. The tubes were put into the incubator at 37° C. for one hour. After having been in the incubator one hour, 1 cubic centimeter of 2 per cent suspension of sheep corpuscles was added to each tube and each tube was well shaken. The tubes were returned to the incubator for one hour and were then removed to room temperature; the results were read about three hours after the corpuscles had been added.

With old serum, or with heated serum, the test was conducted in the following manner: One cubic centimeter of serum was diluted to 5 cubic centimeters with physiologic salt solution, and 0.5 cubic centimeter of the diluted serum was put into each of the ten test tubes. Normal human serum diluted to 0.5 cubic centimeter was added as in the test just described for fresh serum. Antigen and corpuscles were used as already described.

## TEST 1

*Serum preserved by formalin tested by the Wassermann method.*—Specimens 4318, 4319, 4326, and 4328 were secured on September 29, 1915, and the sera were drawn off the clots on September 30, 1915. Each serum was divided into two portions, A and B. Portion A was tested by the Wassermann method without having been mixed with formalin. Portion B was mixed with an equal volume of 1:500 solution of formalin in physiologic salt solution, was kept at room temperature in a cork-stoppered test tube, and was tested at intervals of about a week. A bacteriologic test was made of each serum.

TABLE I.—The influence of formalin on the Wassermann reaction.

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Se- rum.	Am- bo- cep- tor.	Tube—						Result.
							1	2	3	1'	2'	3'	
	1915.		1915.	1915.	cc.	Unit.							
4318	Sept. 29	A	Sept. 30	Sept. 30	0.1	1.0	+	+	tr	+	+	tr	Negative.
		B	Oct. 7	Oct. 7	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	Oct. 12	Oct. 12	0.1	1.0	+	±	0	+	±	0	Do.
4319	---do---	A	Sept. 30	Sept. 30	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	Oct. 7	Oct. 7	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	Oct. 12	Oct. 12	0.1	1.0	+	tr	0	+	tr	0	Do.
4326	---do---	A	Sept. 30	Sept. 30	0.1	1.0	+	0	0	+	+	tr	Strongly positive.
		B	Oct. 7	Oct. 7	0.1	1.0	+	+	tr	+	+	tr	Negative.
		B	Oct. 12	Oct. 12	0.1	1.0	+	tr	0	+	tr	0	Do.
4328	---do---	A	Sept. 30	Sept. 30	0.1	1.0	+	0	0	+	+	tr	Strongly positive.
		B	Oct. 7	Oct. 7	0.1	1.0	+	+	?	0	+	+	Faintly positive.
		B	Oct. 12	Oct. 12	0.1	1.0	+	tr?	0	+	tr	0	Do.

The sign + means complete hæmolysis; ±, hæmolysis between 50 per cent and 100 per cent; tr (trace), hæmolysis less than 50 per cent; +?, barely perceptible sediment; ±? in antigen tube and ± in corresponding control tube, or tr? in antigen tube and tr in corresponding control tube, means very slight difference.



Table I shows that sera 4318 and 4319, which gave negative results with the Wassermann reaction before formalin was added, also gave negative results after formalin had been added. Serum 4326, which before formalin was added gave a strong positive result, gave a negative result on the seventh day and on the twelfth day after the formalin had been added. Serum 4328 gave a strong positive result before formalin was added and a faintly positive result on the seventh day and on the twelfth day after the formalin had been added. All sera were bacteriologically sterile.

## TEST 2

*Serum preserved by formalin tested by the Wassermann method.*—Specimens 4401, 4403, 4404, and 4405 were secured on October 29, 1915. The sera were drawn off the clots on October 30, 1915. Each serum was divided into two portions, A and B. Portion A received no formalin; it was tested by the Wassermann method and by the Tschernogubow modification of the Wassermann reaction on October 30, 1915. Portion B was mixed with an equal volume of 1:500 solution of formalin in physiologic salt solution and was tested on November 7, 1915, by the Wassermann method and by the Tschernogubow modification of the Wassermann reaction before and after it had been heated. Each serum was examined bacteriologically.

TABLE II.—*The influence of formalin on the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Se- rum.	Am- bo- cep- tor.	Tube--						Result.	
							1	2	3	1'	2'	3'		
4401	1915.		1915.	1915.	cc.	Unit.								
	Oct. 29	A	Oct. 30	Oct. 30	0.1	1.0	+	+	0	+	+	tr	Weakly positive.	
		B	-----	Nov. 7	0.1	1.0	+	tr	0	+	±	0	Do,	
B		Nov. 7	---do---	0.1	1.0	+	±	0	+	±	0	Negative.		
4403	---do---	A	Oct. 30	Oct. 30	0.1	1.0	±	0	0	+	+	tr	Strongly positive.	
		B	-----	Nov. 7	0.1	1.0	tr	0	0	±	0	0	Weakly positive.	
		B	Nov. 7	---do---	0.1	1.0	+	tr	0	+	tr	0	Negative.	
4404	---do---	A	Oct. 30	Oct. 30	0.1	1.0	+	tr	0	+	+	0	Moderately posi- tive.	
		B	-----	Nov. 7	0.1	1.0	tr	0	0	±	0	0	Weakly positive.	
		B	Nov. 7	---do---	0.1	1.0	+	tr	0	+	tr	0	Negative.	
4405	---do---	A	Oct. 30	Oct. 30	0.1	1.0	+	±	0	+	+	0	Weakly positive.	
		B	-----	Nov. 7	0.1	1.0	tr	0	0	±	0	0	Do.	
		B	Nov. 7	---do---	0.1	1.0	+	tr	0	+	tr	0	Negative.	

TABLE II'.—*The influence of formalin on the Tschernogubow modification of the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Se- rum.	Tube—										Result.
						1	2	3	4	5	1'	2'	3'	4'	5'	
	1915.		1915.	1915.	cc.											
4401	Oct. 29	A		Oct. 30		+	0	0	0	0	+	+	tr	0	0	Moderately positive.
		B		Nov. 7	0.1	+	+	±	0	0	+	+	+	+	0	Weakly positive.
		B	Nov. 7	...do	0.1	+	+	+	tr	0	+	+	+	+	0	Negative.
4403	...do	A		Oct. 30		0	0	0	0	0	+	+	+	+	0	Strongly positive.
		B		Nov. 7	0.1	+	+	±	0	0	+	+	+	+	0	Weakly positive.
		B	Nov. 7	...do	0.1	+	+	+	0	0	+	+	+	+	0	Negative.
4404	...do	A		Oct. 30		+	0	0	0	0	+	+	+	+	0	Strongly positive.
		B		Nov. 7	0.1	+	+	+	0	0	+	+	+	+	0	Weakly positive.
		B	Nov. 7	...do	0.1	+	+	±	0	0	+	+	+	+	0	Do.
4405	...do	A		Oct. 30		+	+	0	0	0	+	+	+	+	0	Moderately positive.
		B		Nov. 7	0.1	+	+	±	0	0	+	+	+	+	0	Weakly positive.
		B	Nov. 7	...do	0.1	+	+	+	0	0	+	+	+	+	0	Negative.

Tables II and II' show the results obtained by testing formalinized serum by the Wassermann method and by the Tschernogubow modification. Some of these sera gave strongly positive results before formalin had been added. With the unheated sera weakly positive results were obtained a week after the formalin had been added. After having been heated to 55° C. for thirty minutes, all but one serum gave negative results. Unheated, these sera were but slightly anticomplementary a week after having been secured; less so for the Tschernogubow modification than for the Wassermann method. All sera were bacteriologically sterile.

## TEST 3

*The influence of glycerin on the Wassermann reaction.*—Specimens 4318, 4319, 4320, 4321, 4322, 4326, 4328, 4335, 4336, and 4338 were secured on September 29, 1915. On September 30, 1915, the sera were drawn off the clots. Each serum was divided into two portions, A and B. Portion A was tested by the Wassermann method on September 30, 1915, without having been mixed with glycerin. Portion B was mixed with an equal volume of sterilized, neutral glycerin, was kept at room temperature in a cork-stoppered test tube, and was tested at intervals of a week or more. Each serum was examined for bacterial contamination.



TABLE III.—*The influence of glycerin on the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Quantity of serum.	Ambocceptor.	Tube—						Result.
							1	2	3	1'	2'	3'	
4318	1915. Sept. 29	A	Sept. 30	Sept. 30	cc. 0.1	Unit. 1.25	+	+	±	+	+	±	Negative.
	B	Oct. 7	Oct. 7	0.1	1.25	+	+	tr	+	+	tr	Do.	
	B	Oct. 14	Oct. 14	0.1	1.25	+	±	0	+	±	0	Do.	
	B	Oct. 24	Oct. 24	0.1	1.25	+	±	0	+	±	0	Do.	
	B	-----	do	0.1	1.25	0	0	0	0	0	0	Anticomplementary.	
4319	do	A	Sept. 30	Sept. 30	0.1	1.25	+	+	±	+	+	±	Negative.
	B	Oct. 7	Oct. 7	0.1	1.25	+	+	tr	+	+	tr	Do.	
	B	Oct. 14	Oct. 14	0.1	1.25	+	±	0	+	±	0	Do.	
	B	Oct. 24	Oct. 24	0.1	1.25	+	tr	0	+	tr	0	Do.	
	B	-----	do	0.1	1.25	0	0	0	0	0	0	Anticomplementary.	
4320	do	A	Sept. 30	Sept. 30	0.1	1.25	+	+	tr	+	+	tr	Negative.
	B	Oct. 7	Oct. 7	0.1	1.25	+	+	±	+	+	±	Do.	
	B	Oct. 14	Oct. 14	0.1	1.25	+	+	0	+	+	0	Do.	
	B	Oct. 24	Oct. 24	0.1	1.25	+	±	0	+	±	0	Do.	
	B	-----	do	0.1	1.25	0	0	0	0	0	0	Anticomplementary.	
4321	do	A	Sept. 30	Sept. 30	0.1	1.25	+	+	±	+	+	±	Negative.
	B	Oct. 7	Oct. 7	0.1	1.25	+	+	tr	+	+	tr	Do.	
	B	Oct. 14	Oct. 14	0.1	1.25	+	+	0	+	+	0	Do.	
	B	Oct. 24	Oct. 24	0.1	1.25	+	±	0	+	±	0	Do.	
	B	-----	do	0.1	1.25	0	0	0	0	0	0	Anticomplementary.	
4322	do	A	Sept. 30	Sept. 30	0.1	1.25	+	+	±	+	+	±	Negative.
	B	Oct. 7	Oct. 7	0.1	1.25	+	+	tr	+	+	tr	Do.	
	B	Oct. 14	Oct. 14	0.1	1.25	+	+	0	+	+	0	Do.	
	B	Oct. 24	Oct. 24	0.1	1.25	+	±	0	+	±	0	Do.	
	B	-----	do	0.1	1.25	0	0	0	0	0	0	Anticomplementary.	
4326	do	A	Sept. 30	Sept. 30	0.1	1.25	+	0	0	+	+	tr	Strongly positive.
	B	Oct. 7	Oct. 7	0.1	1.25	±	0	0	+	+	0	Do.	
	B	Oct. 14	Oct. 14	0.1	1.25	0	0	0	+	tr	0	Do.	
	B	Oct. 24	Oct. 24	0.1	1.25	0	0	0	+	tr	0	Do.	
	B	-----	do	0.1	1.25	0	0	0	0	0	0	Anticomplementary.	
4328	do	A	Sept. 30	Sept. 30	0.1	1.25	+	±	0	+	+	±	Strongly positive.
	B	Oct. 7	Oct. 7	0.1	1.25	+	tr	0	+	+	tr	Do.	
	B	Oct. 14	Oct. 14	0.1	1.25	tr	0	0	+	tr	0	Do.	
	B	Oct. 24	Oct. 24	0.1	1.25	tr	0	0	+	±	0	Do.	
	B	-----	do	0.1	1.25	0	0	0	0	0	0	Anticomplementary.	
4335	do	A	Sept. 30	Sept. 30	0.1	1.25	+	±	0	+	+	tr	Moderately positive.
	B	Oct. 7	Oct. 7	0.1	1.25	+	±	0	+	+	tr	Do.	
	B	Oct. 14	Oct. 14	0.1	1.25	tr	0	0	+	tr	0	Strongly positive.	
	B	Oct. 24	Oct. 24	0.1	1.25	tr	0	0	+	tr	0	Do.	
	B	-----	do	0.1	1.25	0	0	0	0	0	0	Anticomplementary.	

TABLE III.—*The influence of glycerin on the Wassermann reaction—Continued.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Quantity of serum.	Amboceptor.	Tube—						Result.
							1	2	3	1'	2'	3'	
4336	1915. —do—	A	1915. Sept. 30	1915. Sept. 30	cc. 0.1	Unit. 1.25	+	+	0	+	+	±	Moderately positive.
		B	Oct. 7	Oct. 7	0.1	1.25	+	±	0	+	+	tr	Do.
		B	Oct. 14	Oct. 14	0.1	1.25	tr	0	0	+	tr	0	Strongly positive.
		B	Oct. 24	Oct. 24	0.1	1.25	tr	0	0	+	tr	0	Do.
		B	—do—	—do—	0.1	1.25	0	0	0	0	0	0	Anticomplementary.
4338	—do—	A	Sept. 30	Sept. 30	0.1	1.25	+	±	0	+	+	±	Strongly positive.
		B	Oct. 7	Oct. 7	0.1	1.25	+	0	0	+	+	tr	Do.
		B	Oct. 14	Oct. 14	0.1	1.25	tr	0	0	+	±	0	Do.
		B	Oct. 24	Oct. 24	0.1	1.25	0	0	0	+	tr	0	Do.
		B	—do—	—do—	0.1	1.25	0	0	0	0	0	0	Anticomplementary.

Table III shows the results obtained in testing glycerinated sera 4318, 4319, 4320, 4321, 4322, 4326, 4328, 4335, 4336, and 4338 by the Wassermann method. Glycerin did not noticeably influence this method. The results obtained by this test seven days, fourteen days, and twenty-four days after the sera had been mixed with glycerin were practically identical with the results obtained before the sera had been mixed with glycerin. It seems that sera that gave a positive result while fresh became more strongly positive with age. Negatives remained negative. Unheated, all sera were strongly anticomplementary on the twenty-fourth day after they had been mixed with glycerin; heating the sera to 55° C. for thirty minutes did not entirely destroy the anticomplementary property. All sera remained free from bacterial growth.

## TEST 4

*The influence of glycerin on the Wassermann reaction.*—On October 13, 1915, specimens 4360, 4361, 4364, 4365, 4366, 4369, 4370, 4371, 4372, and 4374 were secured. The sera were drawn off the clots on October 14, 1915. Each serum was divided into two portions, A and B. Portion A, unglycerinated, was tested by the Wassermann method on October 14, 1915. Portion B was mixed with an equal volume of sterilized, neutral glycerin, was kept at room temperature in a cork-stoppered test tube, and was tested by the Wassermann method at intervals of about a week. A bacteriologic examination was made of each serum.





TABLE IV.—*The influence of glycerin on the Wassermann reaction—Continued.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Se- rum.	Am- bo- cep- tor,	Tube—						Result.	
							1	2	3	1'	2'	3'		
4372	1915. Oct. 13	A	1915. Oct. 14	1915. Oct. 14	cc. 0.1	Unit. 1.0	+	+	0	+	+	±	Moderately pos- itive.	
			B	Oct. 24	Oct. 24	0.1	1.0	±	0	0	+	tr	0	Do.
			B	-----	---do---	0.1	1.0	0	0	0	0	0	0	Anticomplemen- tary.
			B	Oct. 31	Oct. 31	0.1	1.0	±	0	0	+	tr	0	Moderately pos- itive.
			B	Nov. 7	Nov. 7	0.1	1.0	±	0	0	+	tr	0	Do.
			A	Oct. 14	Oct. 14	0.1	1.0	+	±	0	+	+	tr	0
4374	---do---	B	Oct. 24	Oct. 24	0.1	1.0	±	0	0	+	tr	0	Do.	
			-----	---do---	0.1	1.0	0	0	0	0	0	0	Anticomplemen- tary.	
			B	Oct. 31	Oct. 31	0.1	1.0	±	0	0	+	tr	0	Moderately pos- itive.
			B	Nov. 7	Nov. 7	0.1	1.0	±	0	0	+	tr	0	Do.
4370	---do---	B	---do---	---do---	0.1	1.5	±	0	0	+	+	+	0	Strongly positive.
4372	---do---	B	---do---	---do---	0.1	1.5	+	tr	0	+	+	+	0	Moderately pos- itive.
4374	---do---	B	---do---	---do---	0.1	1.5	+	0	0	+	+	+	0	Strongly positive.

The result obtained with sera 4360, 4361, 4364, 4365, 4366, 4369, 4370, 4371, 4372, and 4374 by the Wassermann reaction are shown in Table IV. The glycerinated portion of each of these sera gave results practically identical with the result obtained with the nonglycerinated portion. Unheated, nine of these ten sera were strongly anticomplementary on the tenth day, after they had been mixed with glycerin. One serum, No. 4364, had not become anticomplementary. The anticomplementary property was not entirely destroyed by heating the sera to 55° C. for thirty minutes, and it did not alter the result of the test. Such anticomplementary property was overcome by increasing the quantity of amboceptor. All sera were sterile.

## TEST 5

*The influence of glycerin on the Tschernogubow modification of the Wassermann reaction.*—Specimens 4361, 4365, 4366, and 4369 were secured on October 13, 1915. The sera were drawn off the clots on October 14, 1915. Each serum was divided into two portions, A and B. Unheated and unglycerinated, portion A was tested on October 14, 1915. Portion B was mixed with an equal volume of sterilized, neutral glycerin, was kept at room temperature in a cork-stoppered test tube, and was tested two weeks later.



TABLE V.—*The influence of glycerin on the Tschernogubow modification.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Tube—										Result.
						1	2	3	4	5	1'	2'	3'	4'	5'	
	1915.		1915.	1915.	cc.											
4361	Oct. 13	A		Oct. 14		0	0	0	0	0	+	+	+	+	0	Strongly positive.
		B	Oct. 31	Oct. 31	0.1	0	0	0	0	0	+	+	tr	0	0	Do.
4365	do	A		Oct. 14		+	+	±	0	0	+	+	±	0	0	Negative.
		B	Oct. 31	Oct. 31	0.1	+	+	+	0	0	+	+	+	0	0	Do.
4366	do	A		Oct. 14		+	+	+	0	0	+	+	+	0	0	Do.
		B	Oct. 31	Oct. 31	0.1	+	+	+	0	0	+	+	+	0	0	Do.
4369	do	A		Oct. 14		0	0	0	0	0	+	+	+	±	0	Strongly positive.
		B	Oct. 31	Oct. 31	0.1	+	0	0	0	0	+	+	+	0	0	Do.

Table V shows that with these four sera glycerin did not influence the Tschernogubow modification of the Wassermann reaction.

## TEST 6

*The influence of glycerin on the Wassermann reaction.*—Specimens 4394, 4395, 4396, 4397, 4398, and 4399 were secured on October 27, 1915, and the sera were drawn off the clots the next day. Each serum was divided into four portions—A, B, C, and D. Unglycerinated, portion A was heated and tested by the Wassermann method on October 28, 1915. Portion B was mixed with an equal volume of sterilized, neutral glycerin and was heated to 55° C. for thirty minutes on October 28, 1915; was tested by the Wassermann method on November 7, 1915. Portion C was mixed with an equal volume of sterilized, neutral glycerin on October 28, 1915, and was heated and tested by the Wassermann method on November 7, 1915. D was mixed with an equal volume of sterilized, neutral glycerin on October 28, 1915, and without having been heated, was tested by the Wassermann method on November 7, 1915. Each serum was examined bacteriologically.

Table VI shows the results obtained with glycerinated sera 4394, 4395, 4396, 4397, and 4398, tested by the Wassermann method. The glycerin did not noticeably influence the Wassermann reaction. Glycerinated sera that were heated on October 28, 1915, and were tested on November 7, 1915, were not more anticomplementary than were sera that were heated and tested on November 7, 1915. Unheated, five of the six sera were strongly anticomplementary on November 7, 1915. Serum 4399 was no more anticomplementary before having been heated than it was after it had been heated on November 7, 1915.

TABLE VI.—*The influence of glycerin on the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Amboceptor.	Tube—						Result.
							1	2	3	1'	2'	3'	
4394	1915. Oct. 27	A	1915. Oct. 28	1915. Oct. 28	cc. 0.1	Unit. 1.0	+	0	0	+	±	0	Moderately positive.
		B	---do---	Nov. 7	0.1	1.0	±	0	0	+	tr	0	Do.
		C	Nov. 7	---do---	0.1	1.0	tr	0	0	±	tr	0	Do.
		D	-----	---do---	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
4395	---do---	A	Oct. 28	Oct. 28	0.1	1.0	+	±	0	+	±	0	Negative.
		B	---do---	Nov. 7	0.1	1.0	+	tr	0	+	tr	0	Do.
		C	Nov. 7	---do---	0.1	1.0	+	tr	0	+	tr	0	Do.
		D	-----	---do---	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
4396	---do---	A	Oct. 28	Oct. 28	0.1	1.0	+	0	0	+	+	0	Strongly positive.
		B	---do---	Nov. 7	0.1	1.0	tr	0	0	+	tr	0	Do.
		C	Nov. 7	---do---	0.1	1.0	tr	0	0	+	tr	0	Do.
		D	-----	---do---	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
4397	---do---	A	Oct. 28	Oct. 28	0.1	1.0	tr	0	0	+	±	0	Strongly positive.
		B	---do---	Nov. 7	0.1	1.0	0	0	0	+	tr	0	Do.
		C	Nov. 7	---do---	0.1	1.0	0	0	0	+	0	0	Do.
		D	-----	---do---	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
4398	---do---	A	Oct. 28	Oct. 28	0.1	1.0	±	0	0	+	±	0	Strongly positive.
		B	---do---	Nov. 7	0.1	1.0	tr	0	0	+	tr	0	Do.
		C	Nov. 7	---do---	0.1	1.0	tr	0	0	+	tr	0	Do.
		D	-----	---do---	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
4399	---do---	A	Oct. 28	Oct. 28	0.1	1.0	+	±	0	+	±	0	Negative.
		B	---do---	Nov. 7	0.1	1.0	+	tr	0	+	tr	0	Do.
		C	Nov. 7	---do---	0.1	1.0	+	tr	0	+	tr	0	Do.
		D	-----	---do---	0.1	1.0	+	tr	0	+	tr	0	Do.

## TEST 7

*The influence of glycerin on the Tschernogubow modification of the Wassermann reaction.*—Specimens 4394, 4395, and 4396 were secured on October 27, 1915. The sera were drawn off the clots on October 28, 1915. Each serum was divided into three portions—A, B, and C. Portion A, unglycerinated and unheated, was tested by the Tschernogubow modification on October 28, 1915. Portion B was heated to 55° C. for thirty minutes and was then mixed with an equal volume of sterilized, neutral glycerin. On November 7, 1915, portion B was subdivided into portions B and B'. Portion B was tested without further heating, and portion B' was tested after it had been reheated. Unheated, portion C was mixed with an equal volume of sterilized, neutral glycerin on October 28, 1915. On Novem-



ber 7, 1915, portion C was subdivided into two portions, C and C'. Portion C was tested without having been heated, and portion C' was tested after it had been heated to 55° C. for thirty minutes.

TABLE VII.—*The influence of glycerin on the Tschernogubow modification of the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date reheated.	Date tested.	Serum.	Tube—										Result.
							1	2	3	4	5	1'	2'	3'	4'	5'	
4394	Oct. 27	A	1915.	1915.	Oct. 28	cc.	+	0	0	0	0	+	+	+	0	0	Moderately positive.
		B	Oct. 28		Nov. 7	0.1	+	tr	0	0	0	+	+	+	tr	0	Do.
		B'	do	Nov. 7	do	0.1	+	tr	0	0	0	+	+	+	±	0	Do.
		C			do	0.1	tr	0	0	0	0	+	tr	0	0	0	Do.
		C'	Nov. 7		do	0.1	+	tr	0	0	0	+	+	+	±	0	Do.
4395	do	A			Oct. 28		+	+	+	+	0	0	+	+	+	0	Negative.
		B	Oct. 28		Nov. 7	0.1	+	+	+	tr	0	0	+	+	tr	0	Do.
		B'	do	Nov. 7	do	0.1	+	+	+	±	0	0	+	+	±	0	Do.
		C			do	0.1	+	tr	0	0	0	+	tr	0	0	0	Do.
		C'	Nov. 7		do	0.1	+	+	+	±	0	0	+	+	±	0	Do.
4396	do	A			Oct. 28		+	0	0	0	0	+	+	+	+	0	Strongly positive.
		B	Oct. 28		Nov. 7	0.1	+	0	0	0	0	+	+	±	0	0	Do.
		B'	do	Nov. 7	do	0.1	±	0	0	0	0	+	+	±	0	0	Do.
		C			do	0.1	0	0	0	0	0	+	±	0	0	0	Do.
		C'	Nov. 7		do	0.1	+	0	0	0	0	+	+	±	0	0	Do.

As is shown in Table VII, glycerin did not influence the Tschernogubow modification of the Wassermann reaction. Heating the sera to 55° C. for thirty minutes more than once did not alter the results obtained, and unheated, these sera had become but slightly anticomplementary for the Tschernogubow modification of the Wassermann reaction in eleven days.

TEST 8

*The influence of glycerin on the Tschernogubow modification of the Wassermann reaction.*—Specimens 4397, 4398, and 4399 were secured on October 27, 1915. On the next day the sera were drawn off the clots. Each serum was divided into two portions, A and B. Unglycerinated and unheated, portion A was tested by the Tschernogubow modification of the Wassermann reaction on October 28, 1915. Portion B was mixed with an equal volume of sterilized, neutral glycerin and was kept in a cork-stoppered test tube at room temperature. On November 7, 1915, portion B was tested by the Tschernogubow modification before and after the serum had been heated to 55° C. for thirty minutes.

TABLE VIII.—*The influence of glycerin on the Tschernogubow modification of the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Se- rum.	Tube—										Result.
						1	2	3	4	5	1'	2'	3'	4'	5'	
4397	1915. Oct. 27	A	1915.	1915. Oct. 28	cc.	0	0	0	0	0	+	+	+	+	0	Strongly positive.
		B		Nov. 7	0.1	0	0	0	0	0	+	+	+	+	0	Do.
		B	Nov. 7	do	0.1	±	0	0	0	0	+	+	±	0	0	Do.
		A		Oct. 28	0	0	0	0	0	0	+	+	+	0	0	Do.
4398	do	B		Nov. 7	0.1	±	0	0	0	0	+	+	0	0	0	Do.
		B	Nov. 7	do	0.1	+	+	tr	0	0	+	+	+	±	0	Do.
4399	do	A		Oct. 28	+	+	±	0	0	0	+	+	±	0	0	Negative.
		B		Nov. 7	0.1	+	±	0	0	0	+	±	0	0	0	Do.
		B	Nov. 7	do	0.1	+	+	±	0	0	+	+	±	0	0	Do.

Table VIII shows that in test 8 the glycerin did not influence the Tschernogubow modification of the Wassermann reaction. Unheated, these three sera were moderately anticomplementary on November 7, 1915. Heating the sera to 55° C. for thirty minutes destroyed the anticomplementary property.

## TEST 9

*The appearance of anticomplementary properties.*—Specimens 4557, 4558, 4559, 4560, and 4561 were secured on January 4, 1916. On January 5, 1916, the sera were drawn off the clots. Each serum was divided into seven portions—A, B, C, D, E, F, and G. Unglycerinated, portion A was tested by the Wassermann method on January 5, 1916. Portion B was heated to 55° C. for thirty minutes, was mixed with an equal volume of sterilized, neutral glycerin, and was kept at room temperature in a cork-stoppered test tube. Portions C, D, E, F, and G were mixed with equal volumes of sterilized, neutral glycerin and were kept at room temperature in cork-stoppered test tubes. Beginning with January 5, 1916, one portion of glycerinated serum was heated to 55° C. for thirty minutes on every third day until all the portions had been heated. Portion C was heated on January 5; portion D, on January 8; portion E, on January 11; portion F, on January 14; and portion G, on January 16, 1916. On the last day portions B, C, D, E, F, and G were tested by the Wassermann method and a bacteriologic test was made of each serum.



TABLE IX.—The appearance of anticomplementary properties.

No. of serum	Date secured.	Portion.	Date heated.	Date tested.	Se- rum.	Am- bo- cep- tor.	Tube—					Result.	
							1	2	3	1'	2'		3'
4557	1916. Jan. 4	A	1916. Jan. 5	1916. Jan. 5	cc. 0.1	Unit. 1.0	+	+	tr	+	+	tr	Negative.
		B	do	Jan. 16	0.1	1.0	+	+	0	+	+	0	Do.
		C	do	do	0.1	1.0	0	0	0	0	0	0	Anticomplemen- tary.
		D	Jan. 8	do	0.1	1.0	+	tr	0	+	tr	0	Negative.
		E	Jan. 11	do	0.1	1.0	+	tr	0	+	tr	0	Do.
		F	Jan. 14	do	0.1	1.0	+	±	0	+	±	0	Do.
		G	Jan. 16	do	0.1	1.0	+	+	0	+	+	0	Do.
		A	Jan. 5	Jan. 5	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	do	Jan. 16	0.1	1.0	+	+	0	+	+	0	Do.
		C	do	do	0.1	1.0	±	0	0	±	0	0	Do.
4558	do	D	Jan. 8	do	0.1	1.0	+	0	0	+	0	0	Do.
		E	Jan. 11	do	0.1	1.0	+	tr	0	+	tr	0	Do.
		F	Jan. 14	do	0.1	1.0	+	±	0	+	±	0	Do.
		G	Jan. 16	do	0.1	1.0	+	+	0	+	+	0	Do.
		A	Jan. 5	Jan. 5	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	do	Jan. 16	0.1	1.0	+	+	0	+	+	0	Do.
		C	do	do	0.1	1.0	tr	0	0	tr	0	0	Do.
		D	Jan. 8	do	0.1	1.0	+	0	0	+	0	0	Do.
		E	Jan. 11	do	0.1	1.0	+	tr	0	+	tr	0	Do.
		F	Jan. 14	do	0.1	1.0	+	±	0	+	±	0	Do.
4559	do	G	Jan. 16	do	0.1	1.0	+	0	0	+	0	0	Do.
		A	Jan. 5	Jan. 5	0.1	1.0	+	±	0	+	+	tr	Moderately posi- tive.
		B	do	Jan. 16	0.1	1.0	+	0	0	+	+	0	Strongly positive.
		C	do	do	0.1	1.0	0	0	0	+	0	0	Do.
		D	Jan. 8	do	0.1	1.0	0	0	0	+	0	0	Do.
		E	Jan. 11	do	0.1	1.0	0	0	0	+	0	0	Do.
		F	Jan. 14	do	0.1	1.0	0	0	0	+	0	0	Do.
		G	Jan. 16	do	0.1	1.0	0	0	0	+	0	0	Do.
		A	Jan. 5	Jan. 5	0.1	1.0	tr	0	0	+	+	0	Do.
		B	do	Jan. 16	0.1	1.0	0	0	0	+	+	0	Do.
4560	do	C	do	do	0.1	1.0	0	0	0	+	0	0	Do.
		D	Jan. 8	do	0.1	1.0	0	0	0	+	0	0	Do.
		E	Jan. 11	do	0.1	1.0	0	0	0	+	0	0	Do.
		F	Jan. 14	do	0.1	1.0	0	0	0	+	0	0	Do.
		G	Jan. 16	do	0.1	1.0	0	0	0	+	0	0	Do.
		A	Jan. 5	Jan. 5	0.1	1.0	tr	0	0	+	+	0	Do.
		B	do	Jan. 16	0.1	1.0	0	0	0	+	+	0	Do.
		C	do	do	0.1	1.0	0	0	0	+	0	0	Do.
		D	Jan. 8	do	0.1	1.0	0	0	0	+	±	0	Do.
		E	Jan. 11	do	0.1	1.0	0	0	0	+	+	0	Do.
4561	do	F	Jan. 14	do	0.1	1.0	0	0	0	+	+	0	Do.
		G	Jan. 16	do	0.1	1.0	0	0	0	+	+	0	Do.

Table IX shows that fresh sera heated to 55° C. for thirty minutes before they were mixed with glycerin did not become anticomplementary in eleven days, while fresh sera that were heated after they had been mixed with glycerin were anticomplementary eleven days later. Two, Nos. 4559 and 4560, of the five sera tested had become permanently anticomplementary in eleven days; the anticomplementary property was but partially destroyed by heating portions G to 55° C. for thirty minutes. The sera were sterile.

## TEST 9'

*Anticomplementary serum tested.*—Portions C of sera 4557, 4558, 4559, 4560, and 4561 were retested. Instead of 1 unit of amboceptor, 1.5 units were used.

TABLE IX'.—*Moderately anticomplementary sera tested by the Wassermann method.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Amboceptor.	Tube—						Result.
							1	2	3	1'	2'	3'	
	1916.		1916.	1916.	cc.	Unit.							
4557	Jan. 4	C	Jan. 5	Jan. 16	0.1	1.5	+	±	0	+	±	0	Negative.
4558	do	C	do	do	0.1	1.5	+	+	0	+	+	0	Do.
4559	do	C	do	do	0.1	1.5	+	±	0	+	±	0	Do.
4560	do	C	do	do	0.1	1.5	+	0	0	+	+	0	Strongly positive.
4561	do	C	do	do	0.1	1.5	0	0	0	+	+	0	Do.

Table IX' shows that moderate anticomplementary property was readily overcome by increasing the quantity of hæmolytic amboceptor.

## TEST 10

*The anticomplementary property of glycerin.*—Specimens 4595, 4596, 4597, 4598, and 4599 were secured on February 10, 1916. On February 11, 1916, the sera were drawn off the clots. Each serum was divided into two portions, A and B. Both portions were heated to 55° C. for thirty minutes. Portion A was tested by the Wassermann method without having been mixed with glycerin. Portion B was mixed with an equal volume of sterilized, chemically pure glycerin and was tested by the Wassermann method.

TABLE X.—*The anticomplementary property of glycerin mixed with serum.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Amboceptor.	Tube—						Result.
							1	2	3	1'	2'	3'	
	1916.		1916.	1916.	cc.	Unit.							
4595	Feb. 10	A	Feb. 11	Feb. 11	0.1	1.2	+	+	±	+	+	±	Negative.
		B	do	do	0.1	1.2	+	+	0	+	+	0	Do.
4596	do	A	do	do	0.1	1.2	0	0	0	+	+	±	Strongly positive.
		B	do	do	0.1	1.2	0	0	0	+	+	0	Do.
4597	do	A	do	do	0.1	1.2	+	+	±	+	+	±	Negative.
		B	do	do	0.1	1.2	+	+	0	+	+	0	Do.
4598	do	A	do	do	0.1	1.2	+	0	0	+	+	±	Strongly positive.
		B	do	do	0.1	1.2	0	0	0	+	+	0	Do.
4599	do	A	do	do	0.1	1.2	+	tr	0	+	+	±	Do.
		B	do	do	0.1	1.2	+	0	0	+	+	0	Do.

Table X shows that the serum mixed with glycerin was slightly anticomplementary. This anticomplementary property did not affect the result obtained with the test.

#### CONCLUSIONS

Formalin is not a suitable preservative for serum intended for the Wassermann reaction. Sera that gave moderately positive results before formalin was added gave negative results, or nearly negative results, a week after the formalin had been added.

Glycerin kept the sera sterile and did not noticeably influence the Wassermann reaction nor the Tschernogubow modification of the Wassermann reaction.

Unheated, old sera were strongly anticomplementary. Fresh sera that were heated to 55° C. for thirty minutes before they were mixed with glycerin did not become anticomplementary in eleven days.

Fresh sera that were heated to 55° C. after they had been mixed with glycerin were anticomplementary on the eleventh day after the heating.

Nearly all unheated sera that were mixed with glycerin and were kept at room temperature became permanently anticomplementary. Anticomplementary sera could be tested provided the amboceptor was increased.

The anticomplementary property did not alter the result obtained with the Wassermann reaction or with the Tschernogubow modification of the Wassermann reaction.

A mixture of equal parts of glycerin and serum was slightly anticomplementary as compared with the serum alone.





# A CASE OF INFESTATION WITH DIPYLIDIUM CANINUM<sup>1</sup>

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## THREE TEXT FIGURES

It has been my good fortune to find at the autopsy table four chains composed of melon-seedlike tapeworm segments in the small intestine of a male child.

A brief summary of the history is as follows:

José Abad, 8 months old, born in Pila, Laguna Province, residing at Binondo, Manila, was admitted in the department of pediatrics, Philippine General Hospital, on August 21, 1915, on account of enlargement of the abdomen. He is the seventh child of the family and the delivery was normal, although the child had a rather prominent abdomen and was entirely breast-fed.

The present illness has been present since birth. The abdomen was always distended and tympanitic and always retained the enlarged size. The mother, to relieve her child, used to give him an enema of some herb decoction, and after the expulsion of the fecal matter and a good amount of gas, the abdomen would decrease in size and become soft. The child has always been constipated and did not thrive well, although the mother had sufficient milk.

The physical examination showed an underdeveloped and poorly nourished infant with open anterior fontanelle, large mouth due to the habit of sucking the fingers and hands, and large, distended tympanitic abdomen without muscular rigidity or spasm and showing the coils of the large intestine. The peristaltic waves could not be induced by application of cold. No one in the family has the same trouble as this boy.

Stool examination: Negative.

Urine examination: Reaction, acid; sugar, negative; albumen, a decided trace. The microscopic examination showed numerous crystals of acid sodium phosphate, epithelial cells, and some mucus.

TABLE I.—*Examination of the blood.*

Hæmoglobin	80 per cent.
Leucocytes	8,875
Polynuclears	76 per cent.
Small lymphocytes	23 per cent.
Transitionals	1 per cent.

<sup>1</sup> Received for publication January 18, 1916.

The clinical diagnosis was Hirschsprung's disease (megacolon), hydrocele, umbilical hernia, and rachitis.

My report of the autopsy in full, performed on September 10, 1915, is as follows:

The body is that of an underdeveloped and greatly emaciated Filipino male child, measuring 58 centimeters in length and weighing 3.46 kilograms. Rigor mortis and hypostasis are not present. The abdomen is so much dilated and the extremities so small that the body looks like that of a frog. The anterior fontanelle is open, but the sutures are closed. The face has an expression of suffering. The eyelids are open. The pupils cannot be distinguished. The lips are reddened as well as the tongue and buccal mucosa. The thorax is well expanded, but the muscles are poorly developed; the intercostal spaces are depressed, and the costochondral joints are marked. The abdominal wall is very thin and is uniformly distended. In the right lower quadrant there is an egg-shaped mass which is well formed but can be pressed and made to assume different shapes. The coils of the intestines are well marked. On turning the baby to one side, the distention is still present. The inguinal glands are not very much enlarged. The rectum admits the small finger very well.

On section subcutaneous fat is absent. The musculature is very thin. The peritoneum is pinkish, and there are about 20 cubic centimeters of sticky fluid in the peritoneal cavity. All the coils of the intestines are dilated, but the large colon is enormously hypertrophied and dilated. The appendix measures 5 centimeters in length, is under the mesentery of the ileocaecal valve, and contains some faeces. The ascending colon has a slight twist upon its longitudinal axis; it is bluish and large, having a diameter of approximately 3 centimeters. The transverse colon is very much dilated and measures 6 centimeters in diameter. The descending colon measures 4 centimeters in diameter, and the upper part of the sigmoid flexure is about 3 centimeters in diameter, curves toward the right side in a twisted way and is well covered by the peritoneum and by the other portions of the large intestine. The sigmoid and rectum are just as long as the ascending, transverse, and descending parts of the colon. The rectum measures approximately 10 to 12 centimeters in diameter and contains large faeceslike masses; it diminishes in diameter toward the anus. The inferior portion of the rectum has a diameter of 2 centimeters. The walls of the large intestine are very thick and measure about 9 millimeters in thickness. The mesentery of the large intestine has a lower origin than that of the small intestine, and the longest place of the mesentery of the large intestine (or mesosigmoid) measures 11 centimeters. All the blood vessels and lymph channels are very prominent, as well as the mesenteric glands. The small intestine has a thin and congested wall, and nearer the stomach the diameter becomes normal. The stomach is not markedly dilated and is covered by the transverse colon. The liver is small, and the diaphragm reaches the second space on the right side. The omentum is almost absent, and what remains seems to be mucoid tissue.

*Thorax.*—The thymus gland is very small (0.74 gram) and is pinkish yellow.

*Heart.*—The pericardium contains about 3 cubic centimeters of clear, straw-colored, viscid fluid. The heart is dark blue and is small. Foramen ovale is patent, and the orifices of the heart are apparently normal.



The musculature of the heart is opaque, swollen, and dark blue, and the striæ are not visible. Heart weighs 19 grams.

*Lungs.*—The lungs are somewhat smaller than normal, although both crepitate fairly well and float in water; they are pinkish, except their posterior portions, which are dark blue. Both lungs cut easily, and the cut surfaces show a spongelike pinkish substance. The bronchi are free from mucus, and the bronchial glands are not enlarged.

The spleen is small, and foetal lobulations are present. It is firm and cuts with slight resistance. The cut surface is dark blue, and the splenic pulp can be easily scraped away. Malpighian bodies and trabeculæ are not very prominent. The spleen weighs 12 grams.

The adrenals are small and flat. They are firm, brownish yellow, and cut easily; the cortex is firm and is dark yellow. The two adrenals weigh 2.7 grams.

The kidneys are small, firm, and dark blue. The cut surface exudes very much blood; it is dark purple. The cortex is small and looks swollen and glistening; the pyramids are very dark. The Malpighian bodies are not visible. The two kidneys weigh 37 grams.

The liver is small, as already mentioned, and is dark purple; the edges are rounded. It is somewhat firm and cuts with slight resistance. The cut surface exudes much blood, shows a dark purplish color, is glistening, and looks oily. The liver weighs 144 grams.

The stomach contains some fluid with white flocculi. No erosion is found in it. The pyloric opening has a circumference of 2 centimeters. The duodenum is not well tinged with bile.

The gall bladder contains thin golden bile, and its ducts are patent.

The pancreas is reddish pink and does not show any marking.

*Intestines.*—The small intestine contains a reddish mucoid material; it has a thin wall. Toward the upper part of the ileum four chains of tapeworms were found. In the lower part of the intestine was found an isolated segment which seems to be composed of two parts that are united. The superior part enlarges and swells, and later on a small protrusion appears in the middle of it, which elongates and enlarges also, and in this way the segment creeps forward and from place to place. The posterior part does not take any part in this creeping; only it becomes smaller and shorter when the upper part enlarges. Under the microscope this segment shows a rather movable anterior part, but it is not distinctly separated from the rest. The tapeworms that are found have very small bodies and seem to be very young. Examination of the fæces (2 slides) does not show any eggs. The mucosa of the lower part of this intestine is paler and more desquamated than the upper part. The ileocæcal valve is patent, and Peyer's patches above this are all swollen and congested. The large intestine contains grayish, pasty, and sandlike material, and large pieces of this are impacted around small, hard fæces. This material seems to be bismuth. On cleaning the colon, the mucosa is found to be reddened, and in some places minute holes are seen which seem to involve only the mucosa. In the lower part of the rectum there is a large, round, necrotic area, which is approximately 2 centimeters in diameter. Inside this there are some erosions, the bases of which show white material. This is placed opposite the mesenteric attachment. A few centimeters above this there are, also, two longitudinal red ulcers that are transverse to the long axis of the colon. No stenosis nor intussusception can be found in the

large intestine, except that the intestinal wall, especially the muscular layer, looks very thick with prominent and dilated vessels and the lower part contains these ulcers which seem to be very recent. (Patient had no bowel movements twenty-four hours before death, and a Hegar's dilator was introduced into the rectum.)

The prostate gland is apparently normal.

The urinary bladder is apparently normal.

*Throat organs.*—The cervical glands are reddened and slightly enlarged. The thyroid gland is small; otherwise it is apparently normal.

*Brain.*—The cerebrospinal fluid is somewhat increased in amount, and the meninges of the brain are congested; otherwise it is apparently normal.

*Anatomical diagnosis.*—Dilatation and hypertrophy of the colon (megacolon); teniasis (*Dipylidium caninum*); enteritis, catarrhal; ulcerative colitis; passive congestion of the visceral organs; parenchymatous degeneration of the heart, liver, and kidneys; extreme emaciation; rickets; foetal lobulations of the spleen; bismuth impaction in the large intestine; lymphadenitis, mesenteric and cervical; bismuth poisoning (?).

#### IDENTIFICATION OF THE PARASITE

I placed the reddish yellow flat segment on a slide and took it to Mr. L. D. Wharton, instructor in zoölogy, University of the Philippines, who, after seeing the chains, identified them as those of *Dipylidium caninum* and the segment as a proglottid



FIG. 1. *Dipylidium caninum*. a and b, natural size of worm; c and d, natural size of segments; e, the worm enlarged.  $\times 5$ .

of the same species. Later he kindly showed me some of the internal structures of the segments of this parasite.

The segments, as mentioned, look like seeds of a melon with the pointed end of one sticking a little into the rounded end of the segment in front of it (fig. 1). Each one of the large, flat segments has about the middle a shallow groove on either side. The size of each of the segments diminishes toward the head and becomes smaller after staining.

Only one of the parasites has the neck and head, which was buried in the mucosa of the small intestine, so that I had to clip this to preserve the head. This chain has about forty segments and measures after staining 48 millimeters. The head is small, is rhomboid in shape, and under  $\frac{1}{8}$  power and No. 4 objective of a C. Zeiss microscope, it shows the retracted rostellum with the four rows of alternating, rose-thornlike hooklets and two unarmed and somewhat elliptical suckers which hide the other two (fig. 2).

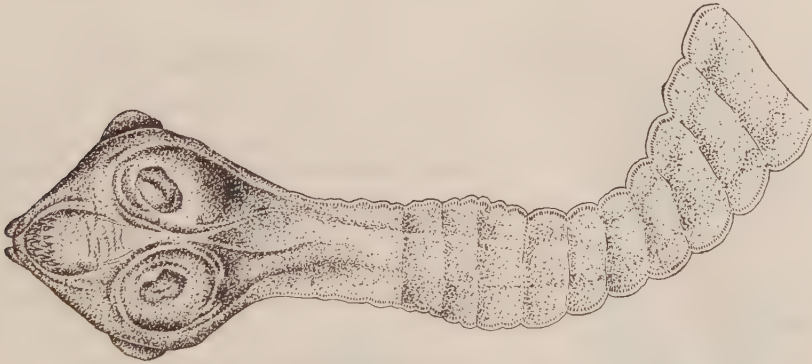


FIG. 2. Head and anterior segments of *Dipylidium caninum*.

The neck is short and does not show marked segmentation. The largest segment is 3 millimeters in length and 1 millimeter in breadth after staining.

The mature segments (fig. 3) have a slight convex outline and show a bilateral arrangement of the reproductive system. The genital pores are double and open about the middle of the segments and opposite each other. The ovaries are bilobed. The vitelline glands lie posterior to the ovaries. The vagina is posterior to the cirrus pouch. The testicles are numerous and are found in the meshes of the reticulum of the uterus.

The vasa deferentia are long, follow a tortuous course, and enter the cirrus pouch which is long and slender.

In the gravid segments the testes and ovaries disappear and the uterus is broken up into capsules which contain the eggs.



The second chain is longer; it is composed of twenty-three segments, which are larger than those of the first. The head is missing, but a part of the neck remains. The chain measures after staining 72 millimeters, and the largest segment is 7 millimeters by 2 millimeters.

The third chain is 2 centimeters long, has no head nor neck, but one end of it is finer and has smaller segments than the other end.

The last chain is composed of five segments and measures 11 millimeters in length after staining.

These parasites are placed in the museum of the College of Medicine and Surgery, University of the Philippines (No. 1495).

#### BIOLOGY OF THE PARASITE

This worm is a parasite of carnivorous animals, accidentally of man and especially of children. It has been classified under Cestoda, family Hymenolepididæ, subfamily Dipylidinae (1) and the description of it has been fully given by C. W. Stiles. (2)

The adults are normally found in the intestines of small dogs (*Canis familiaris*) and cats (*Felis catus*) in great number where they grow rapidly. The ripe proglottides are so active, as shown in this case, that they can pass by their own movements through the anus of the host with the fecal material. Usually the segments are isolated and few, although a great number can come out. As soon as they are out of the anal ring, they crawl toward the hairy parts of the skin, where they deposit

their eggs, which are swallowed by the dog louse (*Trichodectes canis*), or cat louse (*T. rostratus* Nitzsch), or are aspirated by the dog flea (*Ctenocephalis canis* Curtis), cat flea (*C. felis*

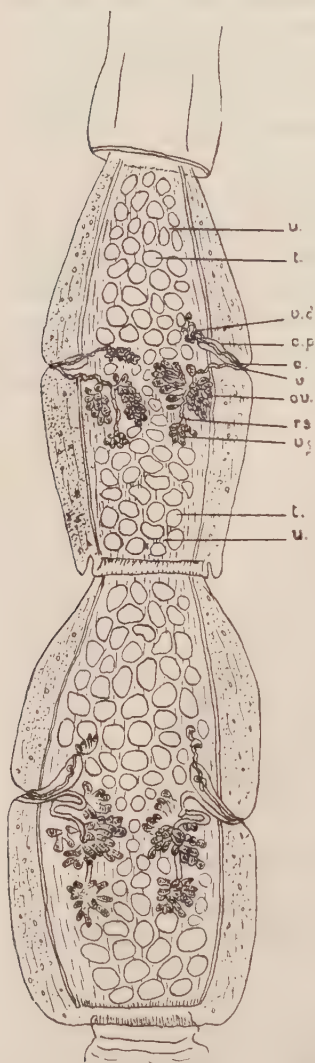


FIG. 3. Posterior segments of *Dipylidium caninum*. u, uterus; t, testes; vd, vas deferens; cp, cirrus pouch; c, cirrus; v, vagina; ov, ovary; rs, receptaculum seminis; vg, vitelline gland.

Bouché), or by that of man (*Pulex irritans* Linnæus). In these insects the eggs develop into cysticeroids (*Cryptoceptes trichodestes* Villot, 1882), as shown by Melnikow in the dog louse and by Grassi in the dog flea.

The dog is infested by biting the louse or flea and may pass the larvæ to man by licking. The cat licks its hair and gets infected or licks the milk that is later on given or taken by a child. This is graphically denounced by R. Blanchard.(3)

Infestation cannot take place directly, according to Riley,(4) from swallowing eggs or segments of the parasite, but only through ingestion of the intermediary host—dog louse and cat flea—as in the other species of *tænia*.

Man may accidentally ingest one of these insects, and the parasites are able then to complete their development in the host. Children are often infested due to their intimate relation with dogs and cats; besides, they do not pay much attention to their food.

*Frequency of these parasites in the dog.*—Bowman(5) and Willets(6) showed the presence of these parasites in the dogs of the Philippines. Krabbe(7) found 78 per cent of dogs and 66 per cent of cats infested in Copenhagen, and Ward(7) found it in from one fifth to four fifths of all dogs examined by various European investigators and says that it is common in Nebraska, in the United States. Wharton found *Dipylidium caninum* in 46.6 per cent of the dogs of Manila.

#### OCCURRENCE IN THE PHILIPPINE ISLANDS

The cestodes are not common intestinal parasites of man, and among them *Dipylidium caninum* is one of the rarest. I have not been able to find any authentic record of it in these Islands, although its probable occurrence has already been foreseen by some workers.

Bowman,(5) in April, 1910, suspected that the Igorots in Benguet subprovince might be infested with this parasite, owing to the fact that dog meat is one of their constant foods. Although he found the fleas *Pulex serraticeps* and *P. irritans* in that region, he did not see any eggs of *Dipylidium caninum* in 100 stool examinations. Nevertheless, he autopsied four dogs and found *Ankylostoma trigonocephalum* (Rud.) and *Dipylidium caninum*.

In October of the same year Willets(6) called the attention of the physicians of these Islands to the probability of human infection with this parasite, for it was common in dogs, and he gave the symptoms and description of the parasite.

The following table of the workers on intestinal parasites in the Philippine Islands will serve for easy comparison:

TABLE II.—*Workers on intestinal parasites in the Philippine Islands.*

Date.	Observer.	Persons infected.	<i>Tænia</i> .	<i>Dipylidium caninum</i> .	Locality.
1901 (8) .....	Strong .....	a 2, 179	2	None .....	Manila.
1902 (9) .....	do .....	b 600	45	.....	Do.
1905 .....	.....	.....	.....	.....	.....
1907 (10) .....	Garrison .....	.....	• 50	None .....	.....
1908 (11) .....	do .....	3, 447	35	do .....	Bilibid Prison.
June-July, 1908 (12) .....	do .....	580	3	do .....	Hospicio de San Juan de Dios.
March-August, 1909.	Gabriel .....	1, 089	6	do .....	Bilibid Prison.
.....	.....	140	.....	do .....	Bureau of Science.
1909 (13) .....	Garrison and Llamas .....	342	1	do .....	Manila.
1909 (14) .....	Garrison, Leynes, and Llamas.	945	.....	.....	Taytay, Rizal.
1910 (15) .....	Rissler and Gomez .....	5, 406	4	None .....	Las Piñas.
1910 .....	do .....	692	17	do .....	Santa Isabel.
1910 .....	do .....	1, 932	32	do .....	Tuguegarao.
1910 (16) .....	Chamberlain, Bloombergh, and Kilbourne.	110	15	(f)	Baguio.
July 1, 1907 (16) .....	do .....	183	9	None .....	Civil Hospital Baguio.
March 3, 1910.	.....	.....	.....	.....	.....
1910 (5) .....	Bowman .....	100	2	do .....	Baguio.
1911 (17) .....	Willets .....	3, 656	59	do .....	Cagayan Valley.
1911 (18) .....	Stitt .....	932	3	do .....	Cavite.
1913 (19) .....	Willets .....	400	1	do .....	Batanes Islands.
1913 (20) .....	Crowell and Hammack .....	c 500	1	do .....	College of Medicine and Surgery.
1913 (21) .....	Tenney .....	250	1	do .....	Philippine Scouts.

a Stools, 1,793; autopsies, 386.

b Stool examinations.

c Autopsies.

d Species.

e Three genera and 5 species.

f One egg supposed to be of *Dipylidium caninum*, but no confirmatory evidence.

Dr. J. D. Jungmann tells me that during his two years' experience in the clinical laboratory of the Philippine General Hospital he has not met with the eggs or segments of this parasite.

Human infestation with *Tænia* is very rare in the Orient, and none of the reports that I could find records this parasite.

TABLE III.—*Showing freedom of man from Tænia and Dipylidium infestation in the Orient.*

Observer.	Stool examinations.	<i>Tænia</i> .	<i>Dipylidium caninum</i> .	Locality.
C. P. Kindleberger (22) ..	7, 768	None .....	None .....	Guam.
Do .....	3, 691	do .....	do .....	.....
T. H. Johnston (23) .....	.....	do .....	do .....	Queensland.
F. Lindsay Woods (24) ..	.....	do .....	do .....	Southern China.
J. Bell (25) .....	850	do .....	do .....	Hongkong.
Alfred Reed (26) .....	120	do .....	do .....	Changsha, China.



In the annual report of the Sanitary Commissioner with the Government of India for 1913 (Calcutta) *Bothriocephalus latus* is recorded in 18 cases, *Tænia asiatica* in 4, *T. saginata* in 21, and *T. solium* in 288, but no case of *Dipylidium* is recorded. Reports from different places in China, published in the China Medical Journal, indicate that *Tænia* infestation is rare and that *Dipylidium caninum* is conspicuous by its absence.

In the article "Medical conditions in the Torrid Zone," the data for which were collected by the College of Medicine and Surgery, University of the Philippines, in 1912, the tapeworm was not found in American Samoa nor in Korea, and 99 per cent of the intestinal worms in China were chiefly *Ascaris*.

In Formosa J. P. Maxwell found only one case of *T. solium* among 15,000 patients.(27)

In the United States of America we have the classical report and description of C. W. Stiles(2) of a child 16 months old at Detroit, Michigan, in 1903, and that of W. A. Riley(4) in 1910 of a boy 11 years old, who was very fond of a bull terrier which was later found to be infested with *Dipylidium caninum*.

In looking for new cases during recent years, the following reports were interesting. Wood(28) reported the intestinal parasites from the different laboratories in the southern part of the United States and found 10 cases of *T. saginata* and 1,004 of *Hymenolepis nana* in 1912.

In 1914 Judkins,(29) from 15,000 stool examinations in Texas, found 71 cases of *Hymenolepis nana*, 67 of *T. saginata*, and 2 of *T. cucumerina*. Of the last he states that they are rare and have no clinical interest, except that children are probably infected from fondling and kissing cats and dogs.

In order to shorten this report, let me quote the various memoirs of Blanchard,(30) before the Academy of Medicine in Paris, 1913, who collected 76 authentic cases and tabulated them according to their geographical distribution.

TABLE IV.—Geographical distribution of cases of *Dipylidium caninum* infestation.

	Cases.		Cases.
France	6	Holland	1
Germany	16	Italy	2
Austria	10	Norway	1
Cape Colony	1	Russia	3
Denmark	21	Sweden	2
United States	2	Switzerland	7
England	3	Venezuela	1

He states that he was unable to find the original papers of a case of Weinland, cited by Monti, of a 6-month-old child,

who expelled the worm with the head spontaneously, and that of Stitt, cited by Stiles and Hassel. I have been especially interested in the latter, for it might refer to the Philippines, but unfortunately I have been also unable to find the original paper.

In Blanchard's collection *Dipylidium caninum* has been found in all ages, but more frequently in children.

TABLE V.—Blanchard's collection of human *Dipylidium caninum* infestation.

Age.	1907	1914	Total.
5 weeks to 6 months .....	20	4	24
7 to 12 months .....	7	1	8
13 to 24 months .....	9	1	10
2 to 3 years .....	2	1	3
9 months to 3 years .....	7	0	7
4 to 8 years .....	6	6	12
9 to 20 years .....	2	2	4
Above 20 years .....	6	2	8
	59	17	76

#### PATHOLOGY

Tapeworms have to fight for the preservation of their species and their lives. To accomplish the first, they are endowed with an enormous capacity to lay eggs. To defend themselves against the action of the digestive juices, they elaborate and excrete an antibody—an antitrypsin (Weinland and Haniel);<sup>(31)</sup> or an antikinase (Dastre and Stessano)<sup>(31)</sup>—a body comparable with that elaborated by the cells of the intestinal mucosa.

That they are also able to secrete offensive substances, Flury<sup>(32)</sup> demonstrated in the case of *Ascaris*, and Blanchard<sup>(3)</sup> states that the burning and itching of the anus during the exit of this worm is due to a toxic substance secreted by this parasite, and that part of the reflex phenomena and especially those disturbances of nutrition as arrest of growth, loss of appetite, and weakness are due to the toxic substance. According to Adami<sup>(31)</sup> these toxic substances diffuse into the tissues and into the blood and bone marrow and other seats of origin of eosinophile cells and stimulate their proliferation and increased production.

In view of the above facts one is tempted to think that, taking for granted that the infestation of my case took place soon after birth, owing to his habit of sucking his fingers, which had been probably licked by a cat or dog, and the exit of the parasite producing much discomfort, chronic constipation and final hypertrophy and dilatation of the colon occurred.

But not all cases of megacolon were found infested with *Dipylidium caninum* as shown by Finney's paper,<sup>(33)</sup> nor have these been found at the autopsies with greater frequency.

As to the lesions they produce, Blanchard<sup>(3)</sup> speaks of the suctorial action of the suckers and cites Schefferdecker who found in some dogs, that had the parasites for a long time, a considerable hypertrophy of the villi, which were from four to five times larger than normal with a rich network of capillaries, and in others the mucosa had true tunnels running longitudinally with two or three worms inside.

#### REASONS FOR ITS INFREQUENCY IN THE PHILIPPINE ISLANDS

The fact that this parasite has been found in dogs (Willets. Bowman, Wharton) and yet not found in man with the frequency that was expected in these Islands is due, I think, to the following reasons:

1. Filipino children, especially those of the lower classes, are as a rule not fond of playing with dogs and cats.
2. Puppies and cats are not usually given milk, but soft boiled rice, in a separate bowl when the inhabitants of the house have finished their meals.
3. Babies are almost always breast-fed, and if given artificial feeding during the later years of infancy they are under the prescription of a physician to avoid the so-called infantile beriberi.
4. Babies in the small huts of the small towns are not left sleeping on the floor, but are placed in hammocks. They are thus little in contact with cats and dogs.
5. Although some tribes eat dog meat, they burn off the hair before removing the skin.

#### PROPHYLAXIS

Prophylaxis is brought about by removing the cats and dogs or freeing them from their intestinal and external parasites. Those who cannot afford to buy cribs should continue the custom of placing the babies in native hammocks, as I suggested before<sup>(34)</sup> and must teach their children not to put their fingers, or anything that they find, into their mouths, as well as not to play with cats and dogs.

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## ILLUSTRATIONS

[Drawings by J. Castro.]

### TEXT FIGURES

- FIG. 1. *Dipylidium caninum*. *a* and *b*, natural size of worm; *c* and *d*, natural size of segments; *e*, the worm enlarged  $\times 5$ .
2. Head and anterior segments of *Dipylidium caninum*. (Zeiss No. 4 objective and  $\frac{1}{8}$  ocular.)
3. Posterior segments of *Dipylidium caninum*. *u*, uterus; *t*, testicles; *vd*, vas deferens; *cp*, cirrus pouch; *c*, cirrus; *v*, vagina; *ov*, ovary, *rs*, receptaculum seminis; *vg*, vitelline gland. (Zeiss No. 4 objective and  $\frac{1}{8}$  ocular.)





## HÆMOLYSIS BY HUMAN SERUM<sup>1</sup>

By E. R. RUEDIGER

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Many different corpuscles have been advocated for the complement-fixation test for syphilis, utilizing the human complement and hæmolytic amboceptor normally present in the serum to be tested, and many different hæmolytic systems have been and still are being employed in the Wassermann reaction and modifications thereof.

For methods that make use of the natural human complement and hæmolytic amboceptor, Hecht<sup>2</sup> used the corpuscles of the sheep. Tschernogubow first (cited by himself) advocated the use of sheep corpuscles. Later he<sup>3</sup> recommended the corpuscles of the guinea pig. Gurd<sup>4</sup> advocates the use of guinea pig corpuscles. The corpuscles of the hen and of other animals have been suggested by other writers.

In conducting the complement-fixation test using alien complement and hæmolytic amboceptor, the sheep hæmolytic system is commonly employed with human serum. The sheep hæmolytic system is not an ideal one to be used with human serum because of the presence of natural antisheep hæmolytic amboceptor in human serum that is readily reactivated by guinea pig complement. Bauer<sup>5</sup> used guinea pig complement, but utilized the antisheep hæmolytic amboceptor normally present in human serum. Noguchi<sup>6</sup> emphatically states that human corpuscles and a corresponding amboceptor must be used in order to get reliable results.

The human hæmolytic system is an ideal one because isohæmolysins practically never exist. The antihuman hæmolytic system is not entirely without faults; animals rarely if ever produce a highly potent antihuman hæmolytic serum, and frequently it is difficult to obtain suitable corpuscles for conducting the tests.

<sup>1</sup> Read before a meeting of the Manila Medical Society, February, 1916. Received for publication October 21, 1915.

<sup>2</sup> *Wien. klin. Wochenschr.* (1908), 21, (1909), 22, 265; (1909), 22, 338.

<sup>3</sup> *Deut. med. Wochenschr.* (1909), 35, 668.

<sup>4</sup> *Journ. Infect. Dis.* (1911), 8, 427.

<sup>5</sup> *Deut. med. Wochenschr.* (1909), 35, 432.

<sup>6</sup> *Journ. Exp. Med.* (1909), 11, 392; *Münch. med. Wochenschr.* (1909), 56, 494.

While the literature contains many references to the presence of natural antishæp hæmolysin in human serum, very little has been done toward overcoming the difficulty by finding a more suitable hæmolytic system. Sachs<sup>7</sup> gives a summary of the work done, and more recently Kolmer and Casselman<sup>8</sup> have more extensively reported on the presence in human serum of hæmolysins for the corpuscles of sheep, dog, ox, goat, hog, rat, chicken, horse, rabbit, and guinea pig. According to the findings of these authors, the hog, rat, chicken, horse, rabbit, or guinea pig hæmolytic systems are to be preferred to the sheep hæmolytic system.

In the following report are recorded the results obtained by testing fifty unheated human sera for hæmolysins against the corpuscles of the sheep, goat, horse, rabbit, and guinea pig and the results obtained by testing fifty reactivated, heated human sera for hæmolysins against the corpuscles of the same animals.

#### HÆMOLYSIS BY UNHEATED HUMAN SERUM

*Technique.*—Fifty human sera were tested within twenty-four hours of the bleeding in the following quantities: 0.4, 0.2, 0.1, 0.05, and 0.025 cubic centimeter. The results obtained with 0.025 cubic centimeters are omitted from the tables because only six sera produced a trace of hæmolysis, while all others gave negative results. To each test tube with serum, enough physiologic salt solution (0.9 per cent) was added to make 1.5 cubic centimeters; corpuscles were added in quantity of 1 cubic centimeter of a 2 per cent suspension, which brought the total quantity in each test tube up to 2.5 cubic centimeters. After each tube had been shaken, it was placed in the incubator at about 37° C. for one hour. During this hour each test tube was shaken at least four times. After an hour in the incubator the tubes were allowed to stand at room temperature (25° C. to 30° C.) and the results were read and recorded about three hours after the corpuscles had been added.

*Hæmolysis of sheep corpuscles.*—Table I shows the results obtained with sheep corpuscles. Sheep-blood corpuscles are readily dissolved by fresh, unheated human serum. None of these fifty human sera failed completely to dissolve the test dose of corpuscles when 0.4 cubic centimeter of serum was used.

<sup>7</sup> Kolle und Wassermann, *Handbuch der pathogenen Microorganismen* (1913), 2, 799.

<sup>8</sup> *Journ. Infect. Dis.* (1915), 16, 441.

TABLE I.—*The effect of unheated human serum on sheep corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				Per cent.
0.4	50	0	0	0
0.2	49	1	0	0
0.1	45	3	2	0
0.05	19	10	8	26

*Hæmolysis of goat corpuscles.*—Table II shows that human serum has fairly good hæmolytic power for the corpuscles of the goat, but not so good as for sheep corpuscles.

TABLE II.—*The effect of human serum on goat corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				Per cent.
0.4	50	0	0	0
0.2	45	3	2	0
0.1	8	15	15	24
0.05	0	0	2	96

*Hæmolysis of horse corpuscles.*—Table III shows that the hæmolytic powers of these fifty unheated human sera for the corpuscles of the horse were approximately equal to those for the corpuscles of the goat.

TABLE III.—*The effect of unheated human serum on horse corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				Per cent.
0.4	49	0	0	2
0.2	44	3	1	4
0.1	20	10	11	18
0.05	1	2	6	82

*Hæmolysis of rabbit corpuscles.*—The hæmolytic power of unheated human serum for rabbit corpuscles was found to be much less than for the corpuscles of the sheep, goat, or horse.



TABLE IV.—The effect of unheated human serum on rabbit corpuscles.

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				Per cent.
0.4	50	0	0	0
0.2	22	11	12	10
0.1	0	0	1	98
0.05	0	0	0	100

*Hæmolysis of guinea pig corpuscles.*—As is shown in Table V, unheated human serum dissolves the corpuscles of the guinea pig almost as readily as it dissolves the corpuscles of the sheep.

TABLE V.—The effect of unheated human serum on guinea pig corpuscles.

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				Per cent.
0.4	50	0	0	0
0.2	47	2	1	0
0.1	19	15	13	6
0.05	1	3	5	82

*Details of results obtained with unheated human serum.*—A detailed record of the results obtained is given in Table VI.

TABLE VI.—Hæmolysis by unheated human serum.

[Numbers represent cubic centimeter of human serum.]

Serum No.	Corpuscles of—															
	Sheep.				Goat.				Horse.				Rabbit.			
	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05
1.....	+	+	+	+	+	+	+	0	+	+	+	0	+	+	0	0
2.....	+	+	+	+	+	+	+	0	+	+	+	tr	+	+	0	0
3.....	+	+	+	+	+	+	±	0	+	+	+	0	+	+	0	0
4.....	+	+	+	+	+	+	±	0	+	+	±	0	+	±	0	0
5.....	+	+	+	+	+	+	±	0	+	+	+	0	+	+	0	0
6.....	+	+	+	±	+	+	±	0	+	+	±	0	+	±	0	0
7.....	+	+	+	tr	+	+	tr	0	+	+	tr	0	+	±	0	0
8.....	+	+	+	0	+	+	tr	0	+	+	tr	0	+	+	0	0
9.....	+	+	+	tr	+	+	±	0	+	+	+	0	+	+	0	0
10.....	+	+	+	±	+	+	±	0	+	+	±	0	+	+	0	0
11.....	+	+	+	±	+	+	±	0	+	+	+	0	+	+	0	0
12.....	+	+	±	0	+	+	0	0	+	±	0	0	+	tr	0	0
13.....	+	+	+	±	+	+	+	0	+	+	+	tr	+	+	tr	0

TABLE VI.—*Hæmolysis by unheated human serum—Continued.*

Serum No.	Corpuscles of—															
	Sheep.				Goat.				Horse.				Rabbit.			
	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05
14.....	+	+	+	±	+	+	tr	0	+	+	±	0	+	+	0	0
15.....	+	+	+	0	+	+	0	0	+	+	0	0	+	tr	0	0
16.....	+	+	+	tr	+	+	tr	0	+	±	0	0	+	+	0	0
17.....	+	+	+	0	+	+	0	0	+	+	tr	0	+	0	0	0
18.....	+	+	+	±	+	+	tr	0	+	+	+	0	+	+	0	0
19.....	+	+	+	+	+	+	+	0	+	+	+	tr	+	+	0	0
20.....	+	+	+	+	+	+	±	0	+	+	+	tr	+	+	0	0
21.....	+	+	+	±	+	+	tr	0	+	+	+	0	+	0	0	0
22.....	+	+	+	+	+	+	±	0	+	+	±	0	+	tr	0	0
23.....	+	+	+	0	+	+	0	0	+	+	tr	0	+	+	0	0
24.....	+	+	+	+	+	+	±	0	+	+	+	0	+	+	0	0
25.....	+	+	+	+	+	+	+	0	+	+	+	±	+	+	0	0
26.....	+	+	+	tr	+	tr	0	0	+	+	tr	0	+	0	0	0
27.....	+	+	+	+	+	+	±	0	+	+	0	0	+	0	0	0
28.....	+	+	+	+	+	+	tr	0	+	+	+	0	+	tr	0	0
29.....	+	+	tr	0	+	±	0	0	+	+	tr	0	+	±	0	0
30.....	+	+	+	+	+	+	±	0	+	+	±	0	+	±	0	0
31.....	+	+	+	±	+	+	tr	0	+	+	tr	0	+	tr	0	0
32.....	+	+	+	±	+	+	tr	0	+	+	tr	0	+	tr	0	0
33.....	+	+	+	tr	+	+	tr	0	+	+	tr	0	+	±	0	0
34.....	+	+	tr	0	+	+	0	0	+	+	+	0	+	tr	0	0
35.....	+	±	0	0	+	tr	0	0	+	+	tr	0	+	tr	0	0
36.....	+	+	+	+	+	+	tr	+	+	+	±	+	+	0	0	0
37.....	+	+	+	tr	+	+	tr	0	+	+	+	tr	+	tr	0	0
38.....	+	+	+	+	+	+	tr	+	+	+	tr	+	±	0	0	0
39.....	+	+	+	tr	+	+	tr	0	+	+	±	0	+	±	0	0
40.....	+	+	+	+	+	+	±	0	+	+	±	0	+	+	0	0
41.....	+	+	+	0	+	+	tr	0	+	+	+	0	+	0	0	0
42.....	+	+	+	+	+	+	+	0	+	+	+	+	+	0	0	0
43.....	+	+	0	0	+	+	0	0	+	+	0	0	+	tr	0	0
44.....	+	+	+	tr	+	+	±	0	+	+	±	0	+	±	0	0
45.....	+	+	±	0	+	±	0	0	+	+	tr	0	+	tr	0	0
46.....	+	+	+	tr	+	+	tr	0	+	0	0	0	+	±	0	0
47.....	+	+	+	+	+	±	0	0	+	tr	0	0	+	+	0	0
48.....	+	+	+	0	+	+	0	0	0	0	0	0	+	tr	0	0
49.....	+	+	+	+	+	+	tr	0	+	±	0	0	+	±	0	0
50.....	+	+	+	±	+	+	±	0	+	+	+	0	+	+	0	0

+ = complete hæmolysis; ± = from 50 to 100 per cent hæmolysis; tr = hæmolysis less than 50 per cent; 0 = no hæmolysis.

Table VI shows that sera 46, 47, 48, 49, and 50 have a low hæmolytic power for horse corpuscles. These five sera were tested at one time, and this drop in the hæmolytic power of these sera did not escape notice. Other things having been equal, the resistance of the corpuscles was at once thought of. As I had never used corpuscles of this horse before, serum 48,

which failed to dissolve the horse corpuscles, was retested against the corpuscles of the same horse and against the corpuscles of three other horses.

TABLE VII.—Serum 48 tested against the corpuscles of four different horses.

Corpuscles of horse—	Human serum.			
	0.4	0.2	0.1	0.05
1	0	0	0	0
2	+	tr	0	0
3	+	±	0	0
4	+	±	0	0

Table VII shows that the corpuscles of horse 1 were unusually resistant toward hæmolysis. While 0.4 cubic centimeter of serum 48 did not produce so much as a trace of hæmolysis with corpuscles of horse 1, the dose of 0.2 cubic centimeter almost completely dissolved the test dose of corpuscles from horses 3 and 4.

#### HÆMOLYSIS BY REACTIVATED, HEATED HUMAN SERUM

*Technique.*—The sera were heated to between 55°C. and 56°C. for thirty minutes, and the heated sera were used in quantities of 0.4, 0.2, and 0.1 cubic centimeter. As complement the pooled sera of five guinea pigs were used in a dose of 0.5 cubic centimeter of a 10 per cent dilution in physiologic salt solution. The natural hæmolytic amboceptor was not removed from the complement serum; it was tested for, and none was found in the test dose for the corpuscles of the sheep, the goat, and the rabbit. With horse corpuscles guinea pig serum produced a trace of hæmolysis. The washed corpuscles were used in dose of 0.5 cubic centimeter of a 4 per cent suspension in physiologic salt solution. Three test tubes were used for each test. In tube 1 were put 0.5 cubic centimeter of diluted, heated human serum representing 0.4 cubic centimeter serum and 0.1 cubic centimeter of physiologic salt solution, 0.5 cubic centimeter of 10 per cent dilution of guinea pig serum, 0.5 cubic centimeter of 4 per cent suspension of washed corpuscles, and 1 cubic centimeter of physiologic salt solution to make the total quantity in the tube 2.5 cubic centimeters. Tube 2 received 0.5 cubic centimeter of diluted serum representing 0.2 cubic centimeter of serum and 0.3 cubic centimeter of physiologic salt solution. Complement, corpuscles suspension, and physiologic salt solution were added as in tube 1.



Tube 3 received 0.5 cubic centimeter of diluted serum representing 0.1 cubic centimeter of serum and 0.4 cubic centimeter of physiologic salt solution. Complement, corpuscles, and salt solution were added as in the foregoing tubes. Each tube was shaken and placed in the incubator at about 37°C. for one hour, during which time the tubes were shaken at intervals of about fifteen minutes. After one hour in the incubator the tubes were removed to the refrigerator at about 7°C., and the results were read on the following morning.

*Hæmolysis of sheep corpuscles.*—Table VIII shows that reactivated, heated human serum readily dissolved the corpuscles of the sheep. All of the fifty sera examined produced some hæmolysis when used in a dose of 0.2 cubic centimeter. Of one serum 0.4 cubic centimeter failed to produce lysis, and of one serum the dose of 0.1 cubic centimeter remained inactive.

TABLE VIII.—*The effect of reactivated, heated human serum on sheep corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				Per cent.
0.4	39	4	6	2
0.2	40	7	3	0
0.1	40	4	5	2

*Hæmolysis of goat corpuscles.*—Table IX shows that reactivated human serum dissolved goat corpuscles almost as readily as it dissolved the corpuscles of the sheep.

TABLE IX.—*The effect of reactivated, heated human serum on goat corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				Per cent.
0.4	30	13	6	2
0.2	31	13	5	2
0.1	23	13	8	2

*Hæmolysis of horse corpuscles.*—The amount of hæmolysis obtained with horse corpuscles was much less than that obtained with the corpuscles of the sheep or of the goat. As is shown in Table X, the dose of 0.2 cubic centimeter of one serum completely dissolved the test dose of corpuscles, and of one serum 0.1 cubic centimeter completely dissolved the corpuscles. Al-

though complete solution of the corpuscles occurred in two instances only, all of the fifty reactivated sera produced some hæmolysis.

TABLE X.—*The effect of reactivated, heated human serum on horse corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				Per cent.
0.4	0	14	36	0
0.2	1	13	36	0
0.1	1	10	39	0

*Hæmolysis of rabbit corpuscles.*—In Table XI are shown the results obtained with rabbit corpuscles. Heated human serum reactivated with guinea pig complement has but weak hæmolytic power for the corpuscles of the rabbit.

TABLE XI.—*The effect of reactivated, heated human serum on rabbit corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				Per cent.
0.4	0	1	9	80
0.2	0	0	8	84
0.1	0	0	8	84

*Hæmolysis of guinea pig corpuscles.*—Table XII shows the effect of heated human serum reactivated with guinea pig complement on guinea pig corpuscles. The hæmolysis obtained was practically negligible. This failure to dissolve guinea pig corpuscles cannot be due to the absence of hæmolytic amboceptor, because in Table V it is shown that unheated human serum readily dissolved guinea pig corpuscles.

TABLE XII.—*The effect of reactivated, heated human serum on guinea pig corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				Per cent.
0.4	0	1	20	58
0.2	0	0	10	80
0.1	0	0	2	96

Table XIII shows the results obtained with the fifty reactivated human sera tested against the corpuscles of sheep, goat, horse, rabbit, and guinea pig. With the corpuscles of the rabbit and of the guinea pig very little hæmolysis was obtained. Although these sera were tested while still fresh, some were decidedly anticomplementary. This anticomplementary property is well shown in sera 1, 2, 5, 26, 28, 32, and 47; less hæmolysis was obtained with the larger quantities of serum than with the smaller quantities. Other sera, such as Nos. 24 and 50, show the anticomplementary property in a minor degree; the large quantities of serum produced no more hæmolysis than did the smaller quantities.

TABLE XIII.—*Hæmolysis by reactivated human serum.*

[Numbers represent cubic centimeter of human serum.]

Serum No.	Corpuscles of—														
	Sheep.			Goat.			Horse.			Rabbit.			Guinea pig.		
	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1
1	±	+	+	±	+	+	tr	tr	tr	0	0	0	0	0	0
2	tr	+	+	±	±	±	tr	tr	tr	0	0	0	0	0	0
3	+	+	+	+	+	+	tr	tr	tr	0	0	0	0	0	0
4	+	+	+	+	+	+	tr	tr	tr	0	0	0	tr	0	0
5	tr	±	±	tr	±	±	tr	tr	tr	0	0	0	0	0	0
6	+	+	+	+	+	+	tr	±	±	0	0	0	0	0	0
7	+	+	+	+	+	+	tr	tr	tr	0	0	0	0	0	0
8	+	+	+	+	+	+	±	±	±	0	0	0	0	0	0
9	+	+	+	+	+	+	±	±	±	0	0	0	tr	0	0
10	+	+	+	+	+	+	tr	tr	tr	0	0	0	0	0	0
11	+	+	+	+	+	+	tr	tr	tr	tr	tr	0	tr	0	0
12	+	+	+	+	+	+	±	±	±	0	0	0	tr	tr	0
13	+	+	+	+	+	+	tr	tr	tr	0	0	0	tr	0	0
14	+	+	+	+	+	+	±	±	±	tr	0	0	0	0	0
15	+	+	+	+	+	+	±	±	tr	0	0	0	tr	tr	0
16	+	+	+	+	+	+	±	±	±	0	0	0	tr	0	0
17	+	+	+	+	+	+	tr	tr	tr	tr	0	0	tr	0	0
18	+	+	+	+	+	+	±	±	±	tr	tr	tr	tr	tr	0
19	+	+	+	+	+	+	tr	tr	tr	0	0	0	0	0	0
20	+	+	+	+	+	+	tr	tr	tr	0	0	0	tr	0	0
21	+	±	tr	+	+	±	tr	tr	tr	0	0	0	tr	tr	0
22	±	±	tr	+	+	±	tr	tr	tr	±	tr	tr	tr	0	0
23	+	+	+	+	+	+	±	±	tr	0	0	0	tr	tr	0
24	±	±	±	±	±	±	tr	tr	tr	0	0	0	0	0	0
25	tr	tr	0	±	±	tr	±	tr	tr	0	0	0	tr	tr	0
26	tr	±	±	tr	±	±	tr	tr	tr	0	0	0	0	0	0
27	+	+	+	+	+	+	tr	tr	tr	0	0	0	tr	tr	tr
28	±	+	+	±	±	±	tr	±	±	0	0	0	tr	0	0
29	+	+	+	±	±	±	tr	tr	tr	0	0	0	0	0	0
30	+	+	+	+	+	+	±	+	+	0	0	0	0	0	0
31	+	+	+	+	+	+	±	±	tr	0	0	tr	±	tr	tr
32	tr	±	±	±	±	±	tr	tr	tr	0	0	0	0	0	0



TABLE XIII.—*Hæmolysis by reactivated human serum*—Continued.

Serum No.	Corpuscles of—														
	Sheep.			Goat.			Horse.			Rabbit.			Guinea pig.		
	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1
33	+	+	+	+	+	+	tr	tr	tr	0	0		0tr	0	0
34	+	+	+	+	+	+	tr	tr	tr	0	0	0	0	0	0
35	+	+	+	+	+	+	tr	tr	tr	tr	tr	tr	tr	tr	0
36	+	+	+	+	+	±	tr	tr	tr	0	0	0	0	0	0
37	+	+	+	±	tr	tr	tr	tr	tr	0	0	0	0	0	0
38	+	±	tr	0	0	0	±	tr	tr	tr	tr	tr	0	0	0
39	+	+	+	+	+	+	±	±	±	0	0	0	0	0	0
40	+	+	+	tr	tr	tr	tr	tr	tr	tr	tr	tr	0	0	0
41	+	+	+	±	±	±	tr	tr	tr	0	0	0	0	0	0
42	+	+	+	±	±	±	tr	tr	tr	tr	tr	tr	0	0	0
43	+	+	+	+	+	+	±	±	±	tr	tr	tr	0	0	0
44	+	+	+	±	±	±	tr	tr	tr	0	0	0	0	0	0
45	+	+	+	tr	tr	tr	tr	tr	tr	0	0	0	0	0	0
46	+	+	+	+	+	+	tr	tr	tr	0	0	0	tr	tr	0
47	0	tr	tr	tr	tr	tr	tr	tr	tr	0	0	0	0	0	0
48	+	+	+	+	±	tr	tr	tr	tr	0	0	0	0	0	0
49	+	+	+	±	tr	tr	tr	tr	tr	0	0	0	tr	0	0
50	tr	tr	tr	tr	tr	tr	tr	tr	tr	0	0	0	0	0	0

+ = complete hæmolysis (100 per cent); ± = hæmolysis between 50 and 100 per cent; tr = hæmolysis less than 50 per cent; 0 = no hæmolysis.

*Reactivation of natural antihorse amboceptor.*—A quantity of fresh human serum was divided into three portions designated as A, B, and C. Portion A was left unheated, portion B was heated to about 55° C. for thirty minutes, and from portion C the natural antihorse amboceptor was removed. Portion C was not heated. The amboceptor was removed in the following manner: One cubic centimeter of unheated serum was diluted with 1 cubic centimeter of physiologic salt solution and was placed in crushed ice. A centrifuge tube with 2 cubic centimeters of washed horse corpuscles was also placed in the crushed ice. Having stood in the crushed ice for fifteen minutes, the diluted serum was poured into the centrifuge tube with the corpuscles; serum and corpuscles were well mixed and were left in the crushed ice. Four centrifuge tubes, each containing 2 cubic centimeters of washed horse corpuscles and 1 cubic centimeter of human serum, were prepared. Two hours after mixing the corpuscles with the serum one tube was centrifuged and the serum was pipetted off and tested for hæmolytic properties. It still contained most of the antihorse amboceptor. At the

end of three hours the second tube was centrifuged, and the serum was pipetted off and tested for hæmolytic power. It still dissolved the horse corpuscles. After five hours the third and fourth tubes were centrifuged, and the serum was pipetted off and tested for hæmolysin. Of this serum 0.4 cubic centimeter still produced a trace of hæmolysis with one half of the usual test dose of horse corpuscles, but 0.2 cubic centimeter of serum mixed with half the usual dose of corpuscles failed to produced hæmolysis.

Now an attempt was made to reactivate the heated human serum (portion B) with human complement (portion C) and with guinea pig complement freed from antihorse amboceptor.

*Technique.*—Three sets of test tubes were prepared. Each set contained three tubes designated as 1, 2, and 3. The first set received unheated human serum (portion A); tube 1 received 0.4 cubic centimeter of serum; tube 2 received 0.2 cubic centimeter of serum, and tube 3 received 0.1 cubic centimeter of serum. Each tube received 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles and enough physiologic salt solution to make the total quantity 2.5 cubic centimeters. In set 2 the heated human serum was reactivated with human complement. Tube 1 received 0.4 cubic centimeter of complement serum, 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles, and enough physiologic salt solution to make the total quantity 2.5 cubic centimeters. Tube 2 received 0.2 cubic centimeter of heated serum, 0.2 cubic centimeter of complement serum, 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles, and enough physiologic salt solution to make the total quantity 2.5 cubic centimeters. Tube 3 received 0.1 cubic centimeter of heated serum, 0.1 cubic centimeter of complement serum, 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles, and enough physiologic salt solution to make the total quantity 2.5 cubic centimeters.

In set 3 the heated serum was reactivated with guinea pig serum freed from natural antihorse amboceptor. Tube 1 received 0.4 cubic centimeter of heated serum and 0.4 cubic centimeter of complement serum. Tube 2 received 0.2 cubic centimeter of heated serum and 0.2 cubic centimeter of complement serum. Tube 3 received 0.1 cubic centimeter of heated serum and 0.1 cubic centimeter of complement serum. To each tube was added 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles and enough physiologic salt solution to make 2.5 cubic centimeters.

TABLE XIV.—*Natural antihorse amboceptor reactivated.*

Set—	Serum.		
	0.4	0.2	0.1
1.....	+	+	±
2.....	+	+	tr
3.....	+	±	0

Table XIV shows that this human serum was readily reactivated with human complement; almost the entire hæmolytic power was restored. Guinea pig complement did not reactivate the serum well.

*Reactivation of the antirabbit amboceptor in fresh human serum.*—A quantity of human serum was divided into three portions—A, B, and C—and was tested for hæmolytic power within five hours after the bleeding. Portion A was not heated; it was tested for the original hæmolytic power. Portions B and C were heated to about 55° C. for thirty minutes and were reactivated with fresh guinea pig serum. The guinea pig serum, also, was tested for hæmolysin for rabbit corpuscles.

*Technique.*—For the human serum three sets of tubes, A, B, and C, were prepared. Each set contained three tubes designated as 1, 2, and 3. Tubes 1, 2, and 3 in set A received 0.4 cubic centimeter, 0.2 cubic centimeter, and 0.1 cubic centimeter of unheated human serum, respectively. To each tube 0.5 cubic centimeter of 4 per cent suspension of rabbit corpuscles was added, and the total quantity in each tube was brought up to 2.5 cubic centimeters with physiologic salt solution. In set B tube 1 received 0.4 cubic centimeter of heated human serum and 0.4 cubic centimeter of guinea pig serum. Tube 2 received 0.2 cubic centimeter of heated human serum and 0.2 cubic centimeter of guinea pig serum, while tube 3 received 0.1 cubic centimeter of heated human serum and 0.1 cubic centimeter of guinea pig serum. To each tube was added 0.5 cubic centimeter of 4 per cent suspension of rabbit corpuscles and enough physiologic salt solution to bring the total quantity up to 2.5 cubic centimeters.

Tubes 1, 2, and 3 in set C received 0.4 cubic centimeter, 0.2 cubic centimeter, and 0.1 cubic centimeter of heated human serum and 0.2 cubic centimeter, 0.1 cubic centimeter, and 0.05 cubic centimeter of guinea pig serum, respectively. Each tube received 0.5 cubic centimeter of 4 per cent suspension of rabbit corpuscles and enough salt solution to bring the total quantity



up to 2.5 cubic centimeters. All tubes were placed in the incubator at 37°C. for one hour and were then removed to room temperature. The results were read and recorded about three hours after the corpuscles had been added.

TABLE XV.—*Natural antirabbit amboceptor reactivated with guinea pig complement.*

Set—	Serum.		
	0.4	0.2	0.1
A -----	+	+	0
B -----	+	+	+
C -----	+	+	±

Table XV shows that the natural antirabbit amboceptor can be reactivated with guinea pig complement provided a sufficiently large dose is used. Equal parts of heated human serum and fresh guinea pig serum had more than double the hæmolytic power of the unheated human serum. Heated human serum mixed with half its volume of fresh guinea pig serum had nearly twice the hæmolytic power of the whole human serum.

*Antirabbit hæmolysin in guinea pig serum.*—Fresh guinea pig serum was tested in doses of 0.8, 0.4, 0.2, and 0.1 cubic centimeter against 0.5 cubic centimeter of 4 per cent suspension of rabbit corpuscles. The total quantity in each was made up to 2.5 cubic centimeters with physiologic salt solution, and the results were read about three hours after the corpuscles had been added.

TABLE XVI.—*Complement control to Table XV. Antirabbit hæmolysis in guinea pig serum.*

Guinea pig serum. cc.	Result.
0.8	±
0.4	tr
0.2	0
0.1	0

As Table XVI shows, this guinea pig serum had slight hæmolytic power for rabbit corpuscles.

*Reactivation of natural antiguinea pig amboceptor.*—In preliminary test it was found that the natural antiguinea pig amboceptor was not entirely absorbed from human serum in two hours; three hours sufficed, while five hours were too long. Human serum that had been in contact with guinea pig cor-

puscles for five hours failed to reactivate the amboceptor. This was probably due to the disappearance of complement.

*Technique.*—A quantity of fresh human serum was divided into three portions designated as A, B, and C. Portion A remained unheated, portion B was heated to 55°C. for thirty minutes, and from portion C the natural antiginea pig amboceptor was removed in the following manner. One cubic centimeter of unheated human serum was diluted with 1 cubic centimeter of physiologic salt solution and was placed in cracked ice. For each tube with diluted serum a centrifuge tube with 3 cubic centimeters of washed guinea pig corpuscles was also placed in the cracked ice. After having stood in the cracked ice for fifteen minutes, the serum was mixed with the corpuscles in the proportion of 2 cubic centimeters of diluted serum to 3 cubic centimeters of corpuscles. These mixtures were allowed to remain in the cracked ice for three hours and were then removed and centrifuged; the clear serum was pipetted off and was used as complement. The complement was carefully tested for amboceptor; the dose of 0.8 cubic centimeter mixed with the test dose of corpuscles failed to produce hæmolysis.

Now it was attempted to reactivate the heated human serum with the human complement and with guinea pig complement.

Three sets of tubes, designated A, B, and B', were prepared. Each set contained four tubes marked 1, 2, 3, and 4. Tubes 1, 2, 3, and 4 of set A received 0.4, 0.2, 0.1, and 0.05 cubic centimeter of serum A, respectively. To each tube was added 0.5 cubic centimeter of 4 per cent suspension of guinea pig corpuscles and enough salt solution to make the total quantity 2.5 cubic centimeters. Set B received serum B and complement C. Tubes 1, 2, 3, and 4 received 0.4, 0.2, 0.1, and 0.05 cubic centimeter of heated serum B and 0.4, 0.2, 0.1, and 0.05 cubic centimeter of complement C, respectively. Each tube received 0.5 cubic centimeter of 4 per cent suspension of guinea pig corpuscles and enough salt solution to bring the total quantity up to 2.5 cubic centimeters. In set B' serum B and guinea pig complement were used. Tubes 1, 2, 3, and 4 received 0.4, 0.2, 0.1, and 0.05 cubic centimeter of heated serum and 0.4, 0.2, 0.1, and 0.05 cubic centimeter of fresh guinea pig serum, respectively. The usual test dose of guinea pig corpuscles was added to each tube, and the total quantity in each tube was brought up to 2.5 cubic centimeters with physiologic salt solution. After shaking, the tubes were put in the incubator at 37°C. for one hour and then removed to room temperature; the results were read about three hours after the corpuscles had been added.

TABLE XVII.—*Reactivation of natural antiguinea pig amboceptor.*

Set—	Human serum.			
	0.4	0.2	0.1	0.05
A .....	+	+	+	±
B .....	+	+	+	tr
B' .....	0	0	0	0

Table XVII shows the results obtained. The unheated human serum had good hæmolytic power for guinea pig corpuscles; complete hæmolysis occurred in tubes 1, 2, and 3 and more than 50 per cent hæmolysis in tube 4. Human complement added to heated human serum almost completely restored the original hæmolytic power. Guinea pig complement did not re-activate the natural antiguinea pig amboceptor.

*The effect of absorption of one amboceptor on another amboceptor.*—After having removed the natural antihorse amboceptor, the serum was tested for hæmolytic power against guinea pig corpuscles. Two sets of tubes, A and B, were used. Each set contained four tubes marked 1, 2, 3, and 4. In set A was tested the hæmolytic power of the whole human serum in quantities of 0.4, 0.2, 0.1, and 0.05 cubic centimeter. Each tube received 0.5 cubic centimeter of 4 per cent suspension of guinea pig corpuscles and enough physiologic salt solution to make the total quantity 2.5 cubic centimeters. In set B the human serum, freed from natural antihorse amboceptor, was titrated against guinea pig corpuscles. All tubes were shaken, placed in the incubator at 37°C. for one hour, and removed to room temperature; the results were read about three hours after the corpuscles had been added.

TABLE XVIII.—*Human serum freed from antihorse amboceptor titrated against guinea pig corpuscles.*

	Serum.			
	0.4	0.2	0.1	0.05
Whole human serum .....	+	+	+	±
Human serum freed from antihorse amboceptor .....	+	+	+	±

Table XVIII shows that removing the antihorse amboceptor did not appreciably disturb the antiguinea pig amboceptor.

*Antihorse amboceptor in serum freed from antiguinea pig amboceptor.*—After having removed the natural antiguinea pig



amboceptor, the serum was tested for hæmolytic power against the corpuscles of the horse. Two sets of tubes, designated as A and B, were used. Each set contained four tubes marked 1, 2, 3, and 4, respectively. In set A the whole human serum was titrated. Tubes 1, 2, 3, and 4 received 0.4, 0.2, 0.1, and 0.05 cubic centimeter of serum, respectively. To each tube was added 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles and enough physiologic salt solution to bring the total quantity up to 2.5 cubic centimeters. In set B the serum freed from antiguinea pig amboceptor was tested for hæmolytic power against horse corpuscles. The serum was used in quantities of 0.4, 0.2, 0.1, and 0.05 cubic centimeter, and horse corpuscles and salt solution were added as in set A. All tubes were shaken, placed in the incubator at 37°C. for one hour, and removed to room temperature; the results were read about three hours after the corpuscles had been added.

TABLE XIX.—*Antihorse amboceptor in serum freed from antiguinea pig amboceptor.*

Set—	Serum.			
	0.4	0.2	0.1	0.05
A .....	+	+	tr	0
B .....	+	+	0	0

Table XIX shows the results obtained. Removing the natural antiguinea pig amboceptor left the natural antihorse amboceptor practically undisturbed.

#### CONCLUSION

Unheated, fresh human serum dissolves the red blood corpuscles of the sheep better than it dissolves those of the guinea pig, goat, horse, or rabbit.

For guinea pig corpuscles the hæmolytic power of fresh, unheated human serum is slightly higher than for goat corpuscles.

The hæmolytic power of fresh, unheated human serum is lower for the corpuscles of the rabbit than for the corpuscles of sheep, goat, horse, or guinea pig.

A fresh, unheated human serum may dissolve the corpuscles of one horse and be inactive against the corpuscles of another horse. The corpuscles differ in resistance toward hæmolysins.

The natural antisheep and antigoaat amboceptors can readily be reactivated with guinea pig complement.

The natural antirabbit amboceptor remained nearly inactive when 0.05 cubic centimeter of guinea pig complement serum was used; larger quantities of guinea pig serum reactivated the amboceptor well.

Guinea pig serum is slightly hæmolytic for rabbit corpuscles.

Guinea pig complement, even when used in large doses, does not readily reactivate the natural antihorse amboceptor. This amboceptor can be readily reactivated with human complement.

Guinea pig complement does not reactivate the natural anti-guinea pig amboceptor.

Natural antiguinea pig amboceptor can readily be reactivated with human complement.

Natural antiguinea pig amboceptor is easily removed from human serum.

Natural antihorse amboceptor is not easily removed from human serum.

Fresh, unheated human serum left in contact with guinea pig corpuscles for more than three hours loses the power to reactivate the antiguinea pig amboceptor.

Natural antihorse amboceptor can be removed from human serum without disturbing the natural antiguinea pig amboceptor.

Natural antiguinea pig amboceptor can be removed from human serum without disturbing the natural antihorse amboceptor.





# AN EXPERIMENTAL STUDY ON THE USE OF APOMORPHINE TO REMOVE FOREIGN BODIES FROM THE RESPIRATORY PASSAGES<sup>1</sup>

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TWO PLATES AND 1 TEXT FIGURE

The use of apomorphine to remove foreign bodies from the respiratory passages is still mentioned in standard textbooks of pharmacology.<sup>2</sup> It is claimed that coincidentally with the act of vomiting caused by apomorphine violent movements of expiration are produced<sup>3</sup> which expel or at least facilitate the expulsion of the foreign body from the respiratory passages. We have not been able to find any reference in the literature that this manner of action of apomorphine has been experimentally established. The present investigation was carried out in order to test whether or not apomorphine exerts such an action.

## THE IRRITABILITY OF THE VOMITING CENTER IN ASPHYXIA

As we are interested in the emetic action of apomorphine when there is obstruction to the respiration, it becomes important first to determine the irritability of the vomiting center in varying degrees of asphyxia. Because of the ready response of the vomiting center to intramuscular injections of apomorphine, dogs were used in all the experiments. Under light ether anæsthesia the tracheal cannula was inserted into the trachea through a short incision in the anterior median line of the neck. The animal was allowed to recover from the influence of anæsthesia. About two hours later different degrees of obstruction to the passage of air into the trachea were produced by

<sup>1</sup> Received for publication January 28, 1916.

<sup>2</sup> Cushny, Text-book of Pharmacology and Therapeutics. 5th ed., Lea & Febiger, Philadelphia & New York (1910), 242. Sollmann, Text-book of Pharmacology. 2d ed., W. B. Saunders Co., Philadelphia & London (1908), 313. Wood, Pharmacology and Therapeutics. J. B. Lippincott Co., Philadelphia & London (1912), 244.

<sup>3</sup> Cushny, loc. cit.

placing a tight clamp on the rubber tubing connected with the free end of the tracheal cannula and by connecting the rubber tube with short pieces of glass tubing whose diameters at one end had been flamed to about 0.9 and 1.5 millimeters, respectively. Apomorphine hydrochloride (0.1 cubic centimeter of a 2 per cent solution per kilogram of body weight) was injected intramuscularly at varying intervals from the commencement of respiratory obstruction. When the occlusion was complete, or when the animals were made to breathe through a circular opening of about 0.9 millimeter in diameter, the vomiting center became quickly paralyzed and apomorphine failed to cause vomiting, even if it was injected at the same time that the respiratory obstruction was accomplished. However, when the animals were made to breathe through a larger opening of approximately 1.5 millimeters in diameter, the vomiting center remained irritable to apomorphine. This observation was not continued longer than two hours, but judging from the behavior of the animals at the end of the observation, the vomiting center would probably have remained irritable even after hours of this degree of asphyxiation. These results are illustrated in the subjoined protocols:

*Protocol 1. November 5, 1915. Male dog, 12.05 kilograms.*

- 10.25 a. m. The dog was put under light ether anæsthesia, the tracheal cannula inserted, the wound closed, and the anæsthesia discontinued.
- 2.55:30 p. m. The dog had recovered from the anæsthesia. A clamp was placed tightly on the rubber tube connected with the tracheal cannula.
- 2.55:50 p. m. Apomorphine hydrochloride (0.1 cubic centimeter of a 2 per cent solution per kilogram) was injected intramuscularly in the gluteal region.
- 2.56 p. m. Retching occurred, and the dog fell to the floor.
- 3.00 p. m. The heartbeat was not palpable.

*Protocol 5. December 21, 1915. Female dog, 5.27 kilograms.*

- 11.45 a. m. The tracheal cannula was inserted, the wound sewed, and etherization discontinued.
- 2.02 p. m. The tracheal cannula was connected to a glass tube with an opening of about 0.9 millimeter in diameter. Apomorphine hydrochloride (0.1 cubic centimeter of a 2 per cent solution per kilogram) was injected intramuscularly.
- 2.04:30 p. m. Retching occurred, and the dog fell to the floor.
- 2.08 p. m. The dog was unconscious, the muscles were relaxed, and the limbs dropped limp when lifted. The femoral pulse was hardly perceptible.

*Protocol 7. December 22, 1915. Male dog, 9.05 kilograms.*

- 11.15 a. m. The tracheal cannula was inserted. The dog was allowed to recover from the ether anæsthesia.
- 1.34:30 p. m. The tracheal cannula was connected to a glass tube with an opening of about 1.5 millimeters in diameter.
- 3.35 p. m. The dog lay quietly on the floor. There was slight dyspnœa, but unnoticeable cyanosis (in the tongue). Apomorphine hydrochloride (0.1 cubic centimeter of a 2 per cent solution per kilogram) was injected intramuscularly.
- 3.36 p. m. Nausea was observed.
- 3.36:30 p. m. The dog vomited. At 3.37, 3.37:30, and 3.38 he vomited again, once each time.
- 3.39 p. m. The dog was chloroformed.

When nonanæsthetized dogs are asphyxiated by shutting off the air from the trachea, retching commonly occurs within the first three minutes without the administration of apomorphine; except in rare cases, the animals die without vomiting. It appears that the vomiting center shares, with the other medullary centers (respiratory, vasomotor, and vagus), the transient stimulation followed by paralysis brought about by asphyxia. In three intramuscular injections of apomorphine hydrochloride (0.1 cubic centimeter of a 2 per cent solution per kilogram of body weight) into three normal dogs, the interval between the injection and the onset of vomiting averaged two minutes and twenty seconds, and in no case was it shorter than two minutes and twelve seconds. The absence of vomiting in experiments 1 and 5 does not necessarily indicate that the vomiting mechanism (center, nerve fibers, nerve endings, or muscles) was paralyzed within two and a half minutes of respiratory obstruction. We have observed in another animal that the femoral pulse was reduced from 78 to 48 beats per minute and became weaker and irregular at the end of the first minute of almost complete tracheal occlusion. It is, therefore, possible that sluggish circulation occurred very early in experiments 1 and 5 and that emesis would have taken place had apomorphine reached the vomiting center in the usual time. Although the vomiting mechanism is readily thrown out of function by complete asphyxia, it is interesting to note that in partial asphyxia, such as is produced by reducing the tracheal opening to 1.5 millimeters in diameter, it not only survives two hours, but it appears more irritable and consequently responds more quickly to apomorphine, as was observed in experiment 7 and in two other experiments.

## WILL THE ADMINISTRATION OF APOMORPHINE FACILITATE THE EXPULSION OF FOREIGN BODIES FROM THE TRACHEA?

Since the vomiting center remains irritable even in the presence of marked respiratory obstruction, we proceeded to test if the administration of apomorphine would facilitate the expulsion of a foreign body from the trachea. With the hope of accomplishing this end, cylinders of reddened agar jelly cut by means of different-sized cork borers were blown with the aid of glass tubing into the trachea of dogs under light ether anæsthesia. The animals were then allowed to come out from under the influence of the anæsthetic and given injections of apomorphine. Seven experiments of this type were performed. One dog died of asphyxia before the administration of apomorphine, and the agar plug, on post-mortem examination, was found at the openings of the two bronchi. Protocol 18 will serve as an illustration of the other six experiments.

*Protocol 18. August 25, 1915. Male dog, 8.05 kilograms.*

- 10.35 a. m. Etherization was commenced.
- 11.02 a. m. An agar cylinder, 6 millimeters in diameter by 40 millimeters long, was blown into the trachea with the aid of a glass tube inserted past the vocal cords.
- 11.03 a. m. The dog was released; he was restless and had moderate dyspnœa.
- 11.04 a. m. A segment of agar cylinder, 7 millimeters long, was expelled by forced expiration.
- 11.06 a. m. The animal was less restless. Respiration was noisy.
- 11.08 a. m. Four cubic centimeters of a 2 per cent solution of apomorphine hydrochloride were injected hypodermically.
- 11.11 a. m. Emesis occurred. The vomitus contained agar cylinder about 10 millimeters long.
- 11.20 a. m. Respiration was still noisy. Chloroform was injected directly into the heart. Autopsy showed no traumatism of the larynx and no agar in the trachea nor bronchi. The remaining segment of the agar was found in the stomach.

From these six experiments it would appear that apomorphine causes expulsion of agar cylinders introduced into the trachea. However, we have not been able to determine that they were still in the trachea at the time of vomiting. In 15 control experiments, where the animals were chloroformed before receiving apomorphine, but still showed difficulty of respiration, the agar was found in the trachea of only two dogs, while in the others it was found in the stomach. The results of these experiments, therefore, are unsatisfactory and not conclusive.



RESPIRATORY PRESSURE AND CONDITION OF THE GLOTTIS DURING  
VOMITING

According to the manner of action ascribed to apomorphine as an agent to remove foreign bodies from the respiratory passages, it must cause a rise of the intrapulmonic pressure and opening of the glottis, or, in other words, a forced expiration during the act of vomiting. The determination, therefore, of the intrapulmonic pressure and the condition of the glottis during emesis should furnish important information relative to the mode of action of apomorphine under consideration.

The intrapulmonic pressure was taken from a tracheal cannula and recorded as follows: One limb of a Y-tube was connected with the tracheal cannula by means of rubber tubing; the second limb was connected with a mercury manometer whose float was adjusted to write on a slow drum; and to the third limb was attached a short piece of rubber tubing so arranged that it could be readily closed or opened when desired. Plate I, fig. 1, is a tracing taken during emesis produced by apomorphine when the third limb of the Y-tube was closed before the beginning of one of the deep inspirations at the commencement of vomiting. The pressure oscillated about the zero line, but in the expulsion time—the time which elapses from the beginning of the convulsive contraction of the abdominal wall to the appearance of vomitus in the mouth—it reached a high level. When the pressure was taken from the perpendicular limb of a T-tube whose horizontal limb was inserted through the median cervical line into the trachea so that it did not interfere with the mechanism of the glottis, the curves obtained were almost identical with Plate I, fig. 1. The curves obtained in this way are illustrated in Plate II, fig. 2. The pressure in this case sank much lower below the zero line during the deep inspirations, and as the rise of pressure was relatively lower, the latter might have been simple rebounds of the mercury in the manometer and not actual increased pressure. This could not, however, be the chief cause; otherwise the waves should progressively diminish. This they did not do. From the standpoint of the mechanism of vomiting, attention is called to the late appearance of the vomitus in the mouth in relation to the beginning of the rise of intrapulmonic pressure. The time relation of these two events seems to indicate that the oesophagus is probably not subjected to a negative pressure

during the passage of vomitus through it as some authors are inclined to believe.<sup>4</sup>

The similarity of the curves, Plate I, fig. 1, and Plate II, fig. 2, affords strong evidence that the glottis closes in the act of vomiting.<sup>5</sup> We have observed, also, when the trachea is connected with a mercury manometer and the dog is allowed to respire through a side tube the terminal opening of which has the combined area of the two nostrils of the animal, that vomiting does not cause a rise of intrapulmonic pressure. The increased pressure noted when the trachea is not occluded, then, must be associated with partial or complete closure of the glottis. On the ingenious suggestion of Prof. R. B. Gibson, of the College of Medicine and Surgery of this University, the condition of the glottis during emesis was further studied as follows: A tracheal cannula was inserted into the trachea—opening directed toward the glottis—and by a Y-tube was connected with a mercury manometer and water-air-pressure pump as shown in fig. 1. If the water is turned on, a current of air flows from the pump through the Y-tube, tracheal cannula, and out of the glottis during quiet respiration, without raising the mercury in the manometer, but if the lumen of the rubber tube connecting the tracheal cannula with the Y-tube be occluded, the current of air is deflected toward the manometer and the mercury rises, falling partially after about four seconds, and then oscillates about this new level till the occlusion is removed. An illustration of this is Plate II, fig. 1. Plate I, fig. 2, shows the curves obtained when the dog vomited. The rise of pressure indicates an obstruction to the passage of air through the naso-laryngo-tracheal passages, and as under the condition of the experiment the dog can accomplish this only by closing the glottis, it serves, therefore, as evidence that this organ closes in the act of vomiting.

The closure of the glottis may persist for ten seconds. Its tracing, when taken simultaneously with that of the intrapulmonic pressure, shows that the glottis stays closed until about the end of vomiting. Whether the glottis opens before or after the increased intrapulmonic pressure has returned to normal, we have not satisfactorily ascertained. The tracings which we could only take on slowly moving drums are not conclusive. However, our objective observation on the movement of air in the trachea

<sup>4</sup> See Howell, *Text-book of Physiology*. 6th ed., W. B. Saunders Co., Philadelphia & London (1915), 736.

<sup>5</sup> "Act of vomiting" is used here and in the following pages to designate the series of events beginning with the first deep inspiration to the appearance of vomitus in the mouth.

during emesis furnishes strong evidence that the glottis remains closed after the increased intrapulmonic pressure has returned to its level before the onset of vomiting. In order to carry on this observation, two cannulae were inserted into the trachea, one toward the glottis and the other toward the lungs. The free ends of the cannulae were joined to a piece of glass tubing, so

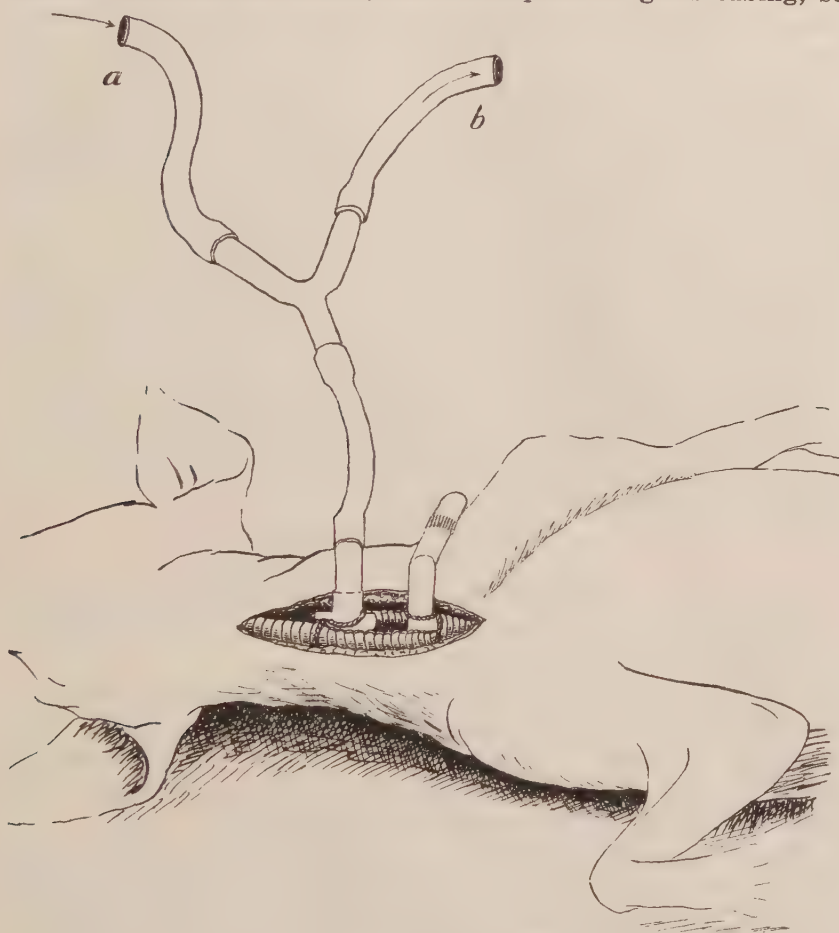


FIG. 1. Shows the connection of the glottis to *b*, the mercury manometer, and to *a*, water-air-pressure pump.

that when the preparation was completed the respired air passed from the lungs through one cannula, through the glass tubing, through the other cannula, and out through the nostrils. During quiet respiration the cork cylinder previously inserted into the glass tubing moved toward the glottis with each expiration and toward the lungs with each inspiration; however, as soon as emesis set in, its movement ceased till the vomitus appeared in



the mouth, at which instant the intrapulmonic pressure must have already fallen to about normal, as Plate I, fig. 1, and Plate II, fig. 2, seem to indicate. When the cork began to move, it did so slowly, and its movement did not exhibit any indication that extra forcible expiration occurred following the act of vomiting.<sup>6</sup> The absence of air current in the respiratory passages during the act of vomiting is shown most decisively if emesis sets in when the cork is about in the middle portion of the glass tube.

The foregoing results point conclusively to the impossibility of removing foreign bodies from the trachea by the use of apomorphine. It seems, moreover, that a foreign body in the respiratory passages below the larynx may, in reality, be driven farther in during the early stage of vomiting because of the descent of the diaphragm and closure of the glottis, which in turn give rise to the rarefaction of the air in the thoracic cavity and a rushing of the air into the deeper portion of the lungs. When tenacious mucous plugs are present in the bronchioles, this may be more than counterbalanced by the stimulating effect of apomorphine on the secretion<sup>7</sup> and peristalsis<sup>8</sup> of the bronchioles, which may loosen and facilitate the expectoration of the plugs after vomiting.

#### SUMMARY AND CONCLUSIONS

Transient stimulation followed by paralysis of the vomiting center occurs when nonanæsthetized dogs are asphyxiated by shutting off the air from the trachea.

Partial asphyxia, such as is produced by reducing the lumen of the trachea to a circular opening of about 1.5 millimeters in diameter, shortens the time required for the emetic action of apomorphine. This is due, presumably, to the increased irritability of the vomiting center to apomorphine.

The intrapulmonic pressure is raised by the convulsive contraction of the abdominal wall which occurs during vomiting, and the rise of pressure seems to begin before the passage of vomitus through the œsophagus.

The glottis remains closed during the act of vomiting, as shown by the method above described. This conclusion is fur-

<sup>6</sup> Cf. Starling, Schäfer's Text-book of Physiology. Young J. Pentland, Edinburg & London, (1898) 2, 325.

<sup>7</sup> Henderson and Taylor, *Journ. Pharmacol. & Expt. Therap.* (1911-12), 2, 153.

<sup>8</sup> Meyer and Gottlieb, *Die Experimentalle Pharmakologie*. Dritte Auflage. Urban & Schwarzenberg, Berlin und Wien (1914), 329.



ther confirmed by the observation that no expiration occurs during the act of vomiting.

A strong expiratory effort is not produced immediately after the expulsion of the vomitus.

The administration of apomorphine cannot facilitate the removal of foreign bodies from the trachea.

#### ACKNOWLEDGMENT

We wish to acknowledge our indebtedness to Prof. R. B. Gibson for reading the manuscript of this paper and for many kind and valuable suggestions made by him during the progress of the experiments.



## ILLUSTRATIONS

### PLATE I

- FIG. 1. Intrapulmonic pressure during apomorphine emesis with the tracheal cannula closed. *C* indicates closure of the third limb of the Y-tube; 1-5, deep inspirations at the beginning of vomiting; 6-7, expulsion time; *O*, opening of the third limb of the Y-tube; *Z*, zero line.
2. Pressure produced by the water-air pump when the dog vomits. 1, beginning of vomiting; 2, end of vomiting; *W-W*, waves due to swallowing of vomitus; *w*, waves due to oscillations of mercury in seeking its normal level; *z*, zero line.

### PLATE II

- FIG. 1. Pressure produced by the water-air pump when the escape of air through the glottis is artificially prevented. 1, occlusion was produced between the tracheal cannula and Y-tube; 2, occlusion removed; *w*, waves due to oscillations of mercury in seeking its normal level; *z*, zero line.
2. Intrapulmonic pressure during apomorphine emesis with the trachea not occluded. 1-8 indicate deep inspirations at the beginning of vomiting; 9, beginning of contraction of abdominal wall; *v*, appearance of vomitus in the mouth.

### TEXT FIGURE

- FIG. 1. Shows the connection of the glottis to *b*, the mercury manometer, and to *a*, the water-air-pressure pump.





DE LA PAZ AND GARCÍA: USE OF APOMORPHINE.]

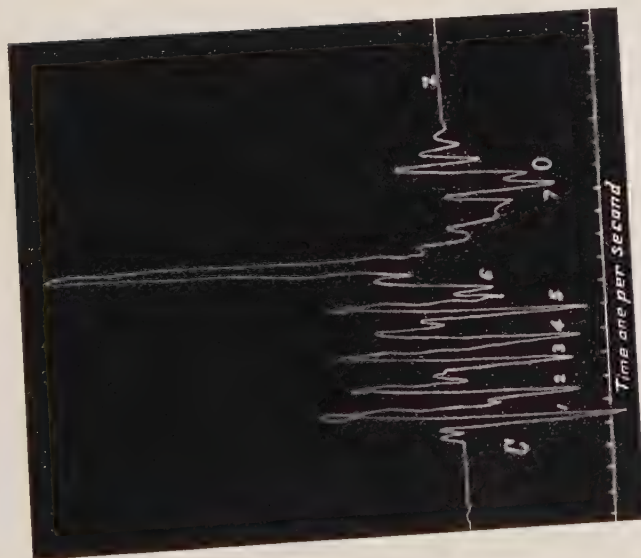


Fig. 1. Intrapulmonic pressure during apomorphine emesis with the tracheal cannula closed. C, indicates closure of the third limb of the Y-tube; 1-5, deep inspirations at the beginning of vomiting; 6-7, expulsion time; O, opening of the third limb of the Y-tube; Z, zero line.

PLATE I.



Fig. 2. Pressure produced by the water-air pump when the dog vomits. 1, beginning of vomiting; 2, end of vomiting; W-W, waves due to swallowing of vomitus; w, waves due to oscillations of mercury in seeking its normal level; Z, zero line.



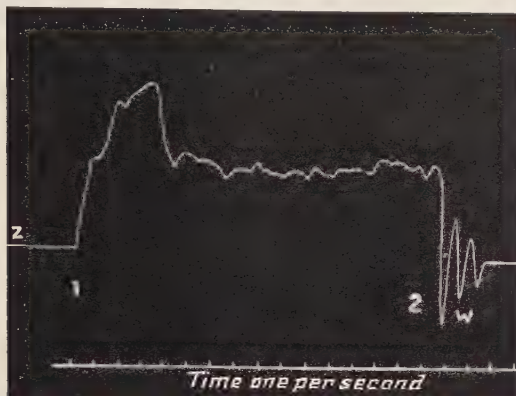


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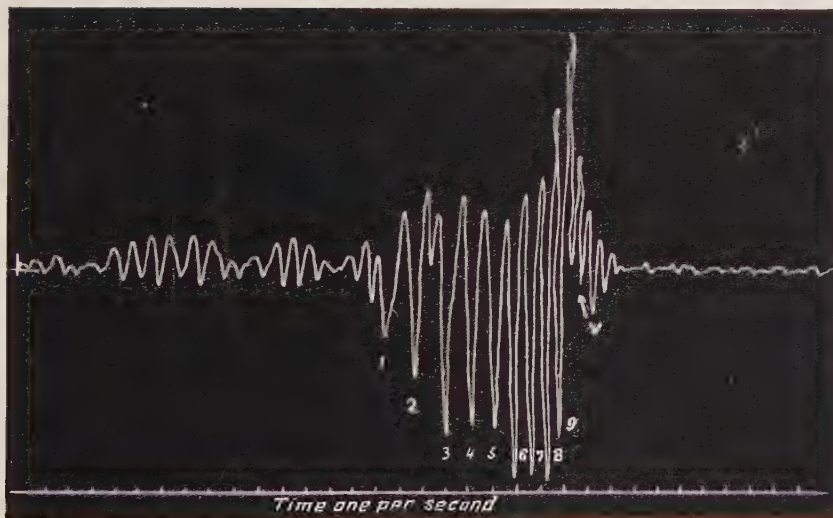


Fig. 2. Intrapulmonic pressure during apomorphine emesis with the trachea not occluded. 1-8 indicate deep inspirations at the beginning of vomiting; 9, beginning of contraction of abdominal wall; v, appearance of vomitus in the mouth.





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BACTERIOLOGICAL EXAMINATIONS OF SWIMMING  
POOLS IN MANILA <sup>1</sup>

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ONE TEXT FIGURE

INTRODUCTION

While it is true that disease is not frequently acquired as a result of infection while bathing, it is of paramount importance that the water used for bathing be free from infectious material. Bacteria such as *Bacillus coli*, *B. typhosus*, *B. diphtheriæ*, *B. dysenteriæ*, *Spirillum cholerae*, *Micrococcus gonorrhæ*, and the various pathogenic organisms found in superficial lesions or occasionally on the normal skin may be disseminated by this means, as may be intestinal parasites belonging to the animal kingdom. Bathers who fail to cleanse themselves properly before entering a pool or those who are carriers and hence disseminators of pathogenic organisms may contaminate the water in the tank.

It has been demonstrated on numerous occasions not only that water has thus been contaminated, but also that bathers have been infected.<sup>2</sup>

Bacterial examinations made of bath water in the prison at Kyoto <sup>3</sup> showed the presence of *B. tuberculosis*, *B. tetani*, gono-

<sup>1</sup> Received for publication March 15, 1916.

<sup>2</sup> Skutsch, *Centralbl. f. Bakt. etc.* (1892), 12, 309; Jäger, *Zeitschr. f. Hyg. u. Infektionskrankh.* (1892), 12, 525; Schultz, *Berl. klin. Wochenschr.* (1899), 36, 865; Fehr, *Ibid.* (1900), 37, 10.

<sup>3</sup> Nakao Abe, *Arch. f. Hyg.* (1908), 65, 140.

coccus, and pneumococcus. The fresh water in the tub (about 160 liters) showed 1,500,000 bacteria per cubic centimeter; after one person bathed in it, the bacterial content was 5,300,000; and after twenty persons used it, 2,086,600,000 bacteria per cubic centimeter were found. In another tank of about 4,800 liters the unused water had 700,000 bacteria per cubic centimeter; after two hundred persons had bathed in the tank, the water had 20,400,000 bacteria per cubic centimeter; after six hundred persons had bathed in it, there were 683,000,000 bacteria per cubic centimeter; and after nine hundred persons had bathed in it, there were 1,799,000,000 bacteria per cubic centimeter.

A series of tests on the waters of certain swimming pools in Germany gave the following results:<sup>4</sup>

POOL A.

Test No.	Date.	Bacteria per cc.
1.....	Jan. 6, 1893	9,700
2.....	Jan. 18, 1893	50,000
3.....	Jan. 25, 1893	75,000

The water of this pool was renewed each day and kept at a temperature of 22°C. Samples for examination were taken from the corner of the basin. In the center of the pool the bacterial content was somewhat greater.

Pool B had 400 cubic meters of water. It was refilled every second day after being well cleaned and was kept at a temperature of 20.6°C. February 2, at 1 o'clock in the afternoon, the water contained 53,500 bacteria per cubic centimeter, after being in the pool for forty hours. February 8, the water contained 22,000 bacteria per cubic centimeter, after being in the pool for fifteen hours.

Pool C contained 180 cubic meters of water. On March 9, at 9 o'clock in the morning, the bacterial content of the pool was 23,000 per cubic centimeter; in the center it was 41,500 per cubic centimeter. On March 16, at 6 o'clock in the afternoon, the bacterial content in the corner of the pool was 6,800 per cubic centimeter; in the center it was 14,000 per cubic centimeter.

Bacteriological examinations of swimming pools in the United States have been comparatively recent and few. Only in comparatively recent years have municipalities established and main-

<sup>4</sup> Max Edel, *Arch. f. Hyg.* (1893), 19, 233.

tained public natatoria, their cost<sup>5</sup> having probably hindered their establishment in larger numbers. Pools maintained by educational institutions have been examined more carefully than the majority of public pools, since the facilities for bacteriological work were near at hand.<sup>6</sup>

The occasional established instances of public-bath infections, together with exaggerated popular accounts of acquired sores, ear troubles, venereal diseases, etc., alleged to be due to infection from contaminated swimming tanks, have caused many to look with aversion upon water that has been in contact with other persons. Since contagious diseases are more prevalent in the tropics than in temperate regions, it is especially important that in countries like the Philippine Islands every possible precaution be taken to prevent the dissemination of infectious material through the medium of bath waters. Much more work has been done on the purification of sewage and of drinking water than of bath water, but fortunately the results of the work on the former are in a great measure applicable to the latter.

Both physical and chemical methods of purification have been employed in attempts to render such waters innocuous. Among the physical methods that have been used to purify water bacteriologically are heat, ultra-violet light, and filtration. These methods have generally been considered too costly for swimming pools. The last gives good results and is used to some extent, but not nearly as much as for drinking water. Heating water to from 60° to 70°C. has been found<sup>7</sup> to give adequate purification.

Many chemical methods have been devised for purifying water. Certain of these methods, through the coagulation of colloids and the formation of precipitates, lessen the turbidity of the water, causing it to appear fresher and cleaner. The use of such substances as mineral acids, alkalies, potassium permanganate, corrosive sublimate, sodium benzoate, boric acid, and ozone has been recommended, but most of them have proved to be too expensive for general use or otherwise im-

<sup>5</sup> Report of the President of the Borough of Manhattan, New York City (1912).

<sup>6</sup> Bunker, J. W. M., *Science* (1910), n. s. 31, 556; Ravenel, *Am. Phy. Ed. Rev.* (1912), 17, 684; Manheimer, W. A., *Pub. Health Rep.* (1915), 30, 2796.

<sup>7</sup> Dunbar, W. P., *Leitfaden f. d. Abwasserreinigungsfrage*. R. Oldenbourg, Berlin (1907), 341.

practical. In practice one of the first substances to be used was quicklime. However, a large amount must be added in order to obtain efficient disinfection, and it often happens that sufficient precipitate is formed greatly to increase the turbidity of the water. Copper sulphate has had various advocates,<sup>8</sup> but while it is very useful when used against plant contaminations such as algæ, it cannot take front rank so far as its bactericidal results are concerned. It, too, has the disadvantage of greatly increasing the turbidity of certain waters.

Chlorine and compounds like hypochlorites, from which chlorine is readily liberated, are comparatively cheap and are much used. They are thought to owe their germicidal action to the liberation of oxygen, thus acting like ozone. They seldom produce undesirable precipitates and have little toxicity. Antiformin has been recommended as a disinfectant by Uhlenhuth and Xylander.<sup>9</sup> Phelps<sup>10</sup> found that the chlorine was not liberated as rapidly from it as from hypochlorite of calcium, but that its use was not more efficient in reducing bacterial content.

Hypochlorite of calcium still seems to be, according to many investigators, the most generally useful disinfectant for sewage and for potable and bath water. Manheimer<sup>11</sup> recommends its use, testing the water from time to time in order to determine that a trace of chlorine is kept constantly present. Bunker<sup>12</sup> found that the addition of bleaching powder in quantities sufficient to give one part available chlorine in 2,000,000 parts of water kept the water sterile for four days with constant use of the pool. He recommended the application of the disinfectant twice a week for the average pool, to insure practically sterile water.

At the University of Wisconsin tank, which has a capacity of 97,000 gallons, Ravenel<sup>13</sup> found that there was a gradual increase in the number of bacteria to the middle of the week, then a decrease, but another increase on Saturday. Treating 250 cubic centimeter samples of water with calcium hypochlorite for thirty minutes, it was found that about 0.5 part of available chlorine per million parts of water materially reduced the bacterial content and usually destroyed the colon bacilli. One

<sup>8</sup> Stokes, *Am. Med.* (1905), 10, 1075; Burrage, *Proc. Ind. Assoc. Sci.* (1909); Rettger and Markey, *Eng. News* (1911), 66, 699.

<sup>9</sup> *Berl. klin. Wochenschr.* (1908), 46, 1346.

<sup>10</sup> Phelps, E. B., *Gesundheits-Ingenieur*. Anklam, Munich (1910), 407.

<sup>11</sup> *Pub. Health Rep.* (1915), 30, 2796.

<sup>12</sup> *Science* (1910), n. s. 31, 556.

<sup>13</sup> *Am. Phy. Ed. Rev.* (1912), 17, 684.



part per million made the water practically sterile. It appeared that the effect of the hypochlorite lasted only about three days, after which there was a considerable increase in bacteria.

When unchlorinated, the water at the University of Pennsylvania showed a continued increase in numbers of bacteria.<sup>14</sup> The tank has a capacity of 155,000 gallons, and city water was used. After adding alum as a coagulant, the water was filtered before it entered the tank. Fifteen hundred gallons were allowed to flow through the tank daily. Every Sunday the tank was emptied, scrubbed, and refilled. *Bacillus pyocyaneus* was frequently isolated from the water. Part of the results obtained is appended herewith.

*Bacteria per cubic centimeter found in University of Pennsylvania swimming tank.*

[Grown 24 hours on agar.]

#### BACTERIA IN UNCHLORINATED WATER.

Day.	Colonies per cc.
1	85
2	340
3	428
4	3,000
5	7,090
6	50,000

On Thursday approximately 0.5 part of available chlorine per million parts of water was added to the water, giving the following results:

#### BACTERIA IN CHLORINATED WATER.

	Colonies per cc.
Before adding chlorine	50,000
15 minutes after adding chlorine	250
2 hours after adding chlorine	0
24 hours after adding chlorine	160

Some of the conclusions drawn from the work just mentioned were that pathogenic organisms may readily find entrance to the water and possibly cause disease; that small amounts of chlorine added to the water in the tank every morning quickly destroy the microorganisms present; that accumulations of hair and other débris at the bottom of the tank should be removed daily by small hand pumps, as they are less readily disinfected than the water.

There are many factors influencing the sterilizing action of chlorine and hypochlorites on water, and a great part of the conflicting evidence found in the literature relative to quantity

<sup>14</sup> W. J. Lyster, *Journ. Am. Med. Assoc.* (1911), 57, 1992.

of disinfectant necessary for efficient purification, time required for sterilization, or duration of bactericidal effect is due to the influence of one or more of these factors. Quality of water, the amount of disinfectant, decomposition rate of the disinfectant, temperature, illumination of the pool, period of time water was in use, extent to which bacteria have gravitated to the bottom, manner of cleaning tank, wind, and other factors modify the biological conditions. Heise<sup>15</sup> has shown that an addition of calcium hypochlorite equivalent to 1 part of available chlorine per million parts of the water used in the Manila swimming pools is reduced in two hours to about 0.1 part.

The bactericidal value of chlorine is reduced<sup>16</sup> by the chlorine-consuming power of various substances with which it may come in contact. Thus Heise<sup>17</sup> found that on adding bleaching powder equivalent to 32 parts of chlorine per million parts of water and allowing it to act two hours in diffused daylight at 28°C. the amount consumed was:

	Parts.
In distilled water	0.75
In city water (unchlorinated)	1 to 2.5
In 200 cc. distilled water + 0.5 cc. saliva	10.0
In 200 cc. distilled water + 0.5 cc. sweat	28.0
In 200 cc. distilled water + 1.0 cc. urine	23.5

The preceding data show that the contamination of a pool by substances such as the secretions and excretions of the human body should be avoided so far as possible, not only because such materials carry bacterial flora with them and in themselves often furnish food material for microorganisms, but also because they actually reduce the amount of available chlorine.

#### POOLS TESTED IN PRESENT WORK

Tests of the bacterial condition of swimming pools have usually been made in temperate zones. On account of the special conditions met with in the tropics, it seemed advisable to make tests on the three swimming pools of Manila. These pools will be designated by the numerals I, II, and III. Pools I and II were made of concrete and were inclosed on the sides. Pool III was lined with glazed tile and was protected by a roof, but was not inclosed on the sides. The size of these tanks is shown in Table A.

<sup>15</sup> *This Journal, Sec. A* (1916), 11, 112.

<sup>16</sup> Glaser, *Arch. f. Hyg.* (1913), 77, 165.

<sup>17</sup> *This Journal, Sec. A* (1916), 11, 114.

TABLE A.—*Approximate dimensions of the swimming pools tested in Manila.*

Pool.	Length.		Width.		Depth.		Capacity.	
	Meters.	Feet.	Meters.	Feet.	Meters.	Feet.	Cubic meters.	Gallons.
I.....	18.3	60	6.0	20	1.2 to 2.4	4 to 8	200	52,400
II.....	18.3	60	5.5	18	1.2 to 2.4	4 to 8	200	52,400
III.....	18.3	60	7.2	23.5	1.2 to 2.7	4 to 9	225	59,400

The city water supply was used by all. This is a river water, which is chlorinated by adding about 0.5 part of available chlorine per million parts of water after passing through a reservoir. The temperatures of the pools were reasonably constant during the period of examination, varying only from one to two degrees from 28.5°C. The water was changed each week on Sunday, except during the week of November 8 to 13, when the water of the previous week continued in use. The disinfectant was applied by first dissolving it in a pail of water and then scattering the solution over the water in the pool. When the bleaching powder was thus properly distributed, no irritant effect on the eyes of bathers or other objectionable features to its use were detected. At pools I and II it was customary simply to drain off the old water and put in the new, while at pool III the emptied tank was thoroughly cleaned before being refilled. The advantage of the latter process in reducing the original bacterial count is strikingly shown in Table I, August 30, where the counts of the uncleaned pools after refilling averaged 68,000 bacteria per cubic centimeter as against 3,600 for the cleaned pool.

#### METHODS USED AND RESULTS OBTAINED

The one hundred eighty-nine samples of the water tested were collected in 50 cubic centimeter, sterile, cotton-stoppered bottles at about 8 o'clock in the morning. At the pool a sample bottle was fastened to a stick, the plug was signed and removed, and the specimen of water was obtained by thrusting the bottle about 1 meter below the surface. These bottles were carried in a covered metallic box, to protect them from dust, rain, and sunlight. The samples were usually plated within forty minutes of the time they were taken.

In this work, unless otherwise stated, the methods and recommendations of the committee on standard methods of water analysis of the American Public Health Association (1912) were

followed, except that meat extract was used in making the media. The fermentation tubes (bouillon or bile) contained 1 per cent of lactose. No peptone was added to the bile. Plates were counted and production of gas was determined after twenty-four hours' incubation. When the presumptive test for *Bacillus coli* was positive, 1 cubic centimeter of the medium in the fermentation tube was plated, lactose litmus agar and Endo medium being used for the confirmatory tests.

Besides these media, which are customarily used to show acid and gas producers, Congo red<sup>18</sup> agar was also tried. This shows the presence of *coli*-like organisms by a darkening of the medium, the acid produced causing a dark blue or black precipitate, which can be more readily seen than the changes of color on lactose litmus agar plates.

This medium was found at times to give positive results when litmus lactose agar did not show the presence of *B. coli*, although the fermentation tube and the subsequent attempts to isolate *B. coli* on litmus lactose agar were positive. Although, as was to be expected, litmus lactose agar plates gave smaller counts at 37°C. than agar at 25°C., Congo red agar at 37°C. gave the highest counts. For the confirmatory tests for *B. coli*, Endo medium seemed to be more suitable than Congo red or litmus lactose agar.

In making the presumptive test for *B. coli*, the bile medium used was found less efficient than lactose bouillon or neutral red lactose bouillon. The fermentation tubes, into which 10 cubic centimeters of the water to be tested were inoculated, contained 30 cubic centimeters of the medium. The results of these tests are summarized in Table XIII.

#### TESTS MADE AND DISCUSSION OF RESULTS

Before trying to determine the effect of disinfectants on the swimming-pool waters, preliminary tests were made to get an idea of the biological condition of the pools and of the variations which occur during use. The results obtained showed that the bacterial content of the swimming pools was not excessive. The plates incubated at blood heat showed a lower count than those kept at the temperature of the room (29°C.). The average count for pool I was highest; this pool had been used by the largest number of persons.

As the tanks were used most extensively in the afternoon, it

<sup>18</sup> Liebermann, L., and Acél, J., *Deutsche Med. Wochenschr.* (1914), 51, 2093.



was thought advisable to determine the variations in bacterial count between the water collected in the morning and that collected in the afternoon.

TABLE B.—*Comparison of morning and afternoon bacterial content of swimming pools.*

[Numbers indicate colonies per cubic centimeter of water.]

Sample taken—	Pool I.				Pool II.			
	Plain agar.	Litmus lactose agar.	Congo red agar.	Average.	Plain agar.	Litmus lactose agar.	Congo red agar.	Average.
Aug. 31 at 8 a. m. -----	2,000	2,500	1,400	2,000	4,800	4,700	3,300	4,300
Aug. 31 at 3 p. m. -----	4,000	10,000	1,800	5,300	40,000	23,000	13,000	25,500
Sept. 4 at 7.30 a. m. -----	100	4,300	2,100	2,200	600	3,500	4,100	2,700
Sept. 4 at 4.10 p. m. -----	3,600	200	1,900	1,900	4,200	4,100	2,200	3,500

From the data in Table B it is seen that the bacterial count in the afternoon is usually higher than early in the morning, when the water has not been stirred up so much by swimmers. The average forenoon count for pools I and II was 2,800 and the average afternoon count was 9,050. For pool III the difference was even greater, the forenoon count being 2,300 and the afternoon count averaging 66,700. A study of the attendance at these pools showed that neither the weekly nor daily variations were sufficiently great seriously to interfere with our work and results. The daily general average of attendance was 32.

The average counts of the three pools on all samples taken before September 20 are given here to exemplify the variations due to different media at the temperatures used.

*Average counts on samples from three pools.*

	Average number of colonies.
Plain nutrient agar, 25° C.	9,500
Litmus lactose agar, 37° C.	9,100
Congo red agar, 37° C.	11,600

The average counts of all samples taken after September 20 on plain agar are 462,290 for 25°C. and 537,445 for 37°C. incubation, the former being about 88 per cent of the latter.

In a number of instances, after samples had been removed for bacteriological tests, a few cubic centimeters of bouillon were added to the bottle of water in order to promote the multiplication of protozoa which might be present. These specimens were examined microscopically, after three days' storage at room temperature.

Similar tests were made on untreated water from the reservoir, and on the chlorinated tap water as drawn at the laboratory, which probably corresponded to the water used in the swimming pools. The results appear in Table C.

TABLE C.—*Protozoa found in water.*

[A, amœbæ; C, ciliates; F, flagellates.]

Date.	Pool—			Reservoir.	Tap.
	I.	II.	III.		
1915.					
Aug. 30	F	F	F	F	ACF
Aug. 31	AF	C	A	F	AF
Sept. 1	A	AF	AC	Negative	A
Sept. 2	A	A	A	F	A
Sept. 3	F	C	A	Negative	AF
Sept. 4	F	A		A	AF
Sept. 6	F	F		Negative	AF
Sept. 7	Negative	Negative		Negative	C
Sept. 8	F	AC		Negative	Negative
Sept. 9	A	AC		F	F
Sept. 10	AC	A		Negative	F
Sept. 11	F	AF		AF	F

These findings seemed to indicate that the swimming-pool waters were no more contaminated with protozoa than the city water supply. As there is not sufficient evidence to show that these are any but harmless organisms, the determinations along this line were discontinued.

In order to ascertain the extent of pollution of the pool, tests for *Bacillus coli* were made for eleven weeks both of the pool waters and of the supply water obtained at the laboratory tap. One cubic centimeter of the water to be tested was inoculated into a fermentation tube. The results of these experiments showed that *B. coli* was only slightly more abundant in these swimming pools than in the city water supply.

Previous to this investigation disinfectants were regularly used twice a week in these tanks. It was found that at the different pools different strengths of disinfectant were being used and applied in different ways. It was desirable to secure the application of the same disinfectant to the three pools in more definite amounts as well as to make chemical tests on the water as the work proceeded. Mr. George W. Heise, chemist, Bureau of Science, coöperated and made all the chemical tests <sup>19</sup>

<sup>19</sup> *This Journal, Sec. A* (1916), 11, 105.

for this work and also supplied the data under "Turbidity" given in Table XIV.

In order to study the effect of various disinfectants on swimming pools, the chemical additions recorded in Table D were made. The Roman numerals refer to the tables of bacteriological data in which the results of the additions enumerated are recorded.

TABLE D.—Outline of tests on Manila swimming pools.

Week.	Disinfectant.			Results in Table—
	Kind.	Quantity (parts per million).	Added—	
1915.				
Aug. 30 to Sept. 4	Chloride of lime		Twice a week	I
Sept. 20 to 25	do	0.5	Once a week	II
Sept. 27 to Oct. 2	do	1.0	do	III
Oct. 4 to 9	do	2.0	do	IV
Oct. 11 to 16	Copper sulphate	1.0	do	V
Oct. 18 to 22	do	2.0	do	VI
Oct. 25 to 30	None	0.0	do	VII
Nov. 1 to 8	Chloride of lime	0.5	Daily	VIII
Nov. 8 to 13 <sup>b</sup>	do	0.5	do	IX
Nov. 15 to 20	Chloride of lime or antiformin.	0.25	do	X
Dec. 6 to 11	Chloride of lime	0.25	do	XI

<sup>a</sup> As available chlorine.

<sup>b</sup> Without change of water.

TABLE I.—Calcium hypochlorite added twice a week to water in swimming pools, week of August 30 to September 4, 1915.

[Bacteria per cubic centimeter of water.]

	Aug. 30.	Aug. 31.	Sept. 1.	Sept. 2.	Sept. 3.	Sept. 4.
Pool I:						
Agar, 25° C	21,500	2,000	2,100	5,000	3,000	1,000
Litmus lactose agar, 37° C	57,000	2,500	3,800	700	100	4,300
Congo red agar, 37° C	50,000	1,400	5,600	600	100	2,100
Average	43,000	2,000	3,800	2,800	1,100	2,200
Pool II:						
Agar, 25° C	69,000	4,800	5,800	1,000	400	600
Litmus lactose agar, 37° C	110,000	4,700	2,200	600	300	3,500
Congo red agar, 37° C	100,000	3,300	4,100	700	100	4,100
Average	93,000	4,300	4,000	800	270	2,700
Pool III:						
Agar, 25° C	2,900	2,000	7,000	5,000	200	
Litmus lactose agar, 37° C	3,000	2,700	3,000	500	900	
Congo red agar, 37° C	5,000	2,100	3,200	800	300	
Average	3,600	2,300	4,800	2,100	500	

TABLE I.—*Calcium hypochlorite added twice a week to water in swimming pools, week of August 30 to September 4, 1915—Continued.*

[Bacteria per cubic centimeter of water—Continued.]

	Aug. 30.	Aug. 31.	Sept. 1.	Sept. 2.	Sept. 3.	Sept. 4.
Pool I:						
<i>Bacillus coli</i> test of 1 cc. water—						
Lactose bile .....	0	+	0	0	0	+
Litmus lactose agar .....		+				+
<i>Coli</i> -like colonies on original plates of—						
Litmus lactose agar .....	0	0	0	0	0	0
Congo red agar .....	0	+	0	0	0	+
Pool II:						
<i>Bacillus coli</i> test of 1 cc. water—						
Lactose bile .....	0	0	0	0	0	0
Litmus lactose agar .....						
<i>Coli</i> -like colonies on original plates of—						
Litmus lactose agar .....	0	0	0	0	0	0
Congo red agar .....	0	0	0	0	0	0
Pool III:						
<i>Bacillus coli</i> test of 1 cc. water—						
Lactose bile .....	+	+	+	+	+	
Litmus lactose agar .....	+	+	+	+	+	
<i>Coli</i> -like colonies on original plates of—						
Litmus lactose agar .....	0	0	0	0	0	
Congo red agar .....	0	+	0	0	0	

TABLE II.—*One-half part per million of available chlorine as calcium hypochlorite, added weekly to water in swimming pools, week of September 20 to 25, 1915.*

[Bacteria per cubic centimeter of water.]

	Sept. 20.	Sept. 21.	Sept. 22.	Sept. 23.	Sept. 24.	Sept. 25.
Pool I:						
Agar, 25° C.....	20	100	10,000	80,000	40,000	100,000
Agar, 37° C.....	50	120	10,500	60,000	60,000	170,000
Average.....	35	110	10,250	70,000	50,000	135,000
Pool II:						
Agar, 25° C.....	3,500	400	12,000	10,000	10,000	110,000
Agar, 37° C.....	4,000	600	13,000	16,000	20,000	150,000
Average.....	3,750	500	12,500	13,000	15,000	130,000
Pool III:						
Agar, 25° C.....	40,000		2,400	100,000	100,000	1,900,000
Agar, 37° C.....	50,000		3,000	100,000	120,000	2,200,000
Average.....	45,000		2,700	100,000	110,000	2,050,000



TABLE III.—*One part per million of available chlorine as calcium hypochlorite, added weekly to water in swimming pools, week of September 27 to October 2, 1915.*

[Bacteria per cubic centimeter of water.]

	Sept. 27.	Sept. 28.	Sept. 29.	Sept. 30.	Oct. 1.	Oct. 2.
Pool I:						
Agar, 25° C.....	900	30	18,000	42,000	120,000	200,000
Agar, 37° C.....	800	60	25,000	200,000	570,000	180,000
Average.....	850	45	21,500	121,000	345,000	190,000
Pool II:						
Agar, 25° C.....	52,000	110	17,000	14,000	22,000	90,000
Agar, 37° C.....	57,000	200	19,000	76,000	68,000	140,000
Average.....	54,500	155	18,000	45,000	45,000	115,000
Pool III:						
Agar, 25° C.....		5,000	190,000	64,000	500,000	170,000
Agar, 37° C.....		7,000	320,000	150,000	630,000	160,000
Average.....		6,000	255,000	107,000	565,000	165,000

TABLE IV.—*Two parts per million of available chlorine as calcium hypochlorite, added weekly to water in swimming pools, week of October 4 to 9, 1915.*

[Bacteria per cubic centimeter of water.]

	Oct. 4.	Oct. 5.	Oct. 6.	Oct. 7.	Oct. 8.	Oct. 9.
Pool I:						
Agar, 25° C.....	4,000	70	2	11,000	1,300,000	150,000
Agar, 37° C.....	3,000	1,000	10	12,000	2,400,000	200,000
Average.....	3,500	550	6	11,500	1,850,000	175,000
Pool II:						
Agar, 25° C.....	1,000	100	22,000	20,000	40,000	200,000
Agar, 37° C.....	2,000	400	27,000	30,000	630,000	240,000
Average.....	1,500	250	24,500	25,000	335,000	220,000
Pool III:						
Agar, 25° C.....	6,000	70	4	55,000	1,200,000	6,000
Agar, 37° C.....	19,000	210	10	45,000	1,270,000	7,000
Average.....	12,500	140	7	50,000	1,235,000	6,500

TABLE V.—*One part per million copper sulphate, added weekly to water in swimming pools, week of October 11 to 16, 1915.*

[Bacteria per cubic centimeter of water.]

	Oct. 11.	Oct. 12.	Oct. 13.	Oct. 14.	Oct. 15.	Oct. 16.
Pool I:						
Agar, 25° C.....	60	2,000	34,000	100,000	400,000	120,000
Agar, 37° C.....	30	1,700	57,000	360,000	500,000	210,000
Average.....	45	1,850	45,000	220,000	450,000	165,000
Pool II:						
Agar, 25° C.....	400	100	3,400	5,100,000	400,000	210,000
Agar, 37° C.....	220	80	17,000	5,700,000	800,000	300,000
Average.....	310	90	10,200	5,400,000	600,000	255,000
Pool III:						
Agar, 25° C.....	22,800	600	1,000	110,000	30,000	-----
Agar, 37° C.....	28,600	110,000	200,000	120,000	80,000	-----
Average.....	26,700	55,300	100,500	115,000	55,000	-----

TABLE VI.—*Two parts per million copper sulphate, added weekly to water in swimming pools, week of October 18 to 23, 1915.*

[Bacteria per cubic centimeter of water.]

	Oct. 18.	Oct. 19.	Oct. 20.	Oct. 21.	Oct. 22.	Oct. 23.
Pool I:						
Agar, 25° C.....	500	28,000	170,000	1,800,000	5,100,000	17,000,000
Agar, 37° C.....	600	18,000	220,000	2,800,000	6,300,000	18,000,000
Average.....	550	23,000	195,000	2,300,000	5,700,000	17,500,000
Pool II:						
Agar, 25° C.....	900	20,000	570,000	2,300,000	5,400,000	-----
Agar, 37° C.....	12,000	19,000	280,000	3,900,000	6,900,000	-----
Average.....	10,500	19,500	425,000	3,100,000	6,150,000	-----
Pool III:						
Agar, 25° C.....	120,000	160,000	20,000	300	3,000	2,800,000
Agar, 37° C.....	130,000	150,000	50,000	9,000	4,000	4,800,000
Average.....	125,000	155,000	35,000	4,650	3,500	3,800,000

TABLE VII.—*No disinfectant added to water in swimming pools, week of October 25 to 30, 1915.*

[Bacteria per cubic centimeter of water.]

	Oct. 25.	Oct. 26.	Oct. 27.	Oct. 28.	Oct. 29.	Oct. 30.
Pool I:						
Agar, 25° C.....	60	1,600	12,000	240,000	80,000	380,000
Agar, 37° C.....	500	17,000	16,000	300,000	100,000	720,000
Average.....	280	9,300	14,000	270,000	90,000	550,000
Pool II:						
Agar, 25° C.....	50	28,000	100,000	120,000	500,000	800,000
Agar, 37° C.....	1,000	40,000	120,000	180,000	600,000	1,400,000
Average.....	525	34,000	110,000	150,000	550,000	1,100,000
Pool III:						
Agar, 25° C.....			19,000	40,000	90,000	940,000
Agar, 37° C.....			29,000	100,000	200,000	1,500,000
Average.....			24,000	70,000	145,000	1,220,000

TABLE VIII.—*One-half part per million of available chlorine as calcium hypochlorite, added daily to water in swimming pools, week of November 1 to 6, 1915.*

[Bacteria per cubic centimeter of water.]

	Nov. 1.	Nov. 2.	Nov. 3.	Nov. 4.	Nov. 5.	Nov. 6.
Pool I:						
Agar, 25° C.....	100	30	60	0	80	0
Agar, 37° C.....	200	100	80	40	300	6
Average.....	150	65	70	20	190	3
Pool III:						
Agar, 25° C.....	30,000		40	170	100	
Agar, 37° C.....	340,000		100	280	560	500
Average.....			70	225	330	

TABLE IX.—*One-half part per million of available chlorine as calcium hypochlorite, added daily to water in swimming pools, week of November 8 to 13, 1915. Second week in use.*

[Bacteria per cubic centimeter of water.]

	Nov. 8.	Nov. 9.	Nov. 10.	Nov. 11.	Nov. 12.	Nov. 13.
Pool I:						
Agar, 25° C.....	20,000	2,700	60	30	200	10
Agar, 37° C.....	23,000	9,600	400	170	10,000	300
Average.....	21,500	6,150	230	100	5,100	155
Pool III:						
Agar, 25° C.....	68,000	400	40	2,300	30	100
Agar, 37° C.....	60,000	1,000	800	5,700	1,600	600
Average.....	64,000	700	420	4,000	815	350

TABLE X.—One-half part per million of available chlorine as calcium hypochlorite, added daily to pool III. One-seventh part per million of available chlorine as antiformin, added daily to pool I. Week of November 15 to 20, 1915.

[Bacteria per cubic centimeter of water.]

	Nov. 15.	Nov. 16.	Nov. 17.	Nov. 18.	Nov. 19.	Nov. 20.
Pool I:						
Agar, 25° C.....	1,300	1,500	500	1,000	16,000	20,500
Agar, 37° C.....	2,400	19,000	10,000	4,000	34,000	42,000
Average.....	1,850	10,000	5,250	2,500	25,000	31,250
Pool III:						
Agar, 25° C.....	400	3,000	220	300	30	16
Agar, 37° C.....	800	5,100	7,100	2,000	80	20
Average.....	600	4,050	3,700	1,150	55	18

TABLE XI.—One-fourth part per million of available chlorine as calcium hypochlorite, added daily to water in swimming pools, week of December 6 to 11, 1915.

[Bacteria per cubic centimeter of water.]

	Dec. 6.	Dec. 7.	Dec. 8.	Dec. 9.	Dec. 10.	Dec. 11.
Pool I:						
Agar, 25° C.....	20	10	30	10	20	160
Agar, 37° C.....	160	30	210	100	380	390
Average.....	90	20	120	55	200	275
Pool III:						
Agar, 25° C.....	60	50	180	2,700	1,200	29,000
Agar, 37° C.....	240	600	2,900	23,500	13,000	44,000
Average.....	150	325	1,500	15,600	12,500	36,500

TABLE XII.—Average bacterial counts of pools.

[Bacteria per cubic centimeter of water.]

25° INCUBATION.

Table.	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
II.....	1,760	250	8,130	63,300	50,000	703,000
III.....	26,450	1,713	75,000	40,000	214,000	153,300
IV.....	3,670	80	7,330	28,670	846,700	118,700
V.....	7,750	900	12,800	1,770,000	276,670	165,000
VI.....	700	24,000	370,000	2,050,000	5,250,000	17,000,000
VII.....	55	14,800	43,330	133,330	223,330	706,670
VIII.....	100	30	50	85	90	0
IX.....	44,000	1,550	50	1,165	115	55
X { I.....	1,300	1,500	500	1,000	16,000	20,500
III.....	400	3,000	220	300	30	16
XI.....	40	30	105	1,355	610	14,580
Average.....	7,838	4,350	47,046	371,745	625,231	1,716,529



TABLE XII.—Average bacterial counts of pools—Continued.  
37° INCUBATION.

Table.	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
II.....	4,025	360	5,500	53,600	66,600	846,600
III.....	28,900	2,420	121,300	142,000	422,600	160,000
IV.....	8,000	537	9,009	29,000	1,433,300	147,000
V.....	9,600	37,260	91,300	2,060,000	460,000	255,560
VI.....	6,300	18,500	250,000	3,350,000	6,600,000	18,000,000
VII.....	750	28,500	55,000	193,330	300,000	1,040,000
VIII.....	200	100	90	160	430	253
IX.....	41,500	5,300	600	2,935	5,800	450
X { I.....	2,400	19,000	10,000	4,000	34,000	42,000
II.....	800	5,100	7,100	2,000	80	20
XI.....	200	315	1,555	14,300	6,690	22,195
Average.....	9,334	10,672	50,131	441,484	848,136	1,864,916

TABLE XIII.—Summary of tests for *Bacillus coli* in swimming-pool waters.

Table.	Disinfectant used in pool.			<i>Bacillus coli</i> , times present during one week.							
	Kind.	Quantity (parts per million).	Frequency.	Pool I.		Pool II.		Pool III.		Average.	
				1 cc.		1 cc.		1 cc.		1 cc.	
				10 cc.	10 cc.	10 cc.	10 cc.	10 cc.	10 cc.	10 cc.	10 cc.
I	Calcium hypochlorite.....	-----	Twice a week.	2	-----	0	-----	5	-----	2.23	-----
II	Chlorine from calcium hypochlorite.	0.5	Weekly....	1	4	1	4	1	2	1	3.33
III	do.....	1.0	do.....	0	4	0	4	2	4	0.66	4
IV	do.....	2.0	do.....	0	4	0	3	0	4	0	3.66
V	Copper sulphate.....	1.0	do.....	0	3	0	2	0	2	0	2.33
VI	do.....	2.0	do.....	0	2	0	0	0	0	0	0.66
VII	None.....	-----	-----	1	4	1	3	0	2	0.66	3
VIII	Chlorine from calcium hypochlorite.	0.5	Daily.....	0	1	-----	-----	0	3	0	2
IX	Chlorine from calcium hypochlorite (2d week).	0.5	do.....	1	1	-----	-----	2	2	2.5	2.5
X	Chlorine from calcium hypochlorite.	0.5	do.....	-----	-----	-----	-----	0	0	0	0
X	Chlorine from antiformin.....	†	do.....	2	2	-----	-----	-----	2	2	2
XI	Chlorine from calcium hypochlorite.	0.25	do.....	0	3	-----	-----	1	3	0.5	3

## DISCUSSION OF TABLES

A study of the preceding tables shows the relative restraining effects on *Bacillus coli* of the different disinfectants used. During week I calcium hypochlorite was used, in the same manner as used previous to these tests, but the strength of the disinfectant was low, and adequate purification was not effected. During week II, when, at the beginning of the week, 0.5 part of available chlorine by chemical analysis per million parts of water was added in the form of calcium hypochlorite, the *B. coli*

count was lowered. This was reduced still further during week III, when 1 part of chlorine per million was used, and during week IV, when 2 parts of chlorine were applied. The results of the determinations for *B. coli* are not shown in Tables III to XI, but are summarized in Table XIII. Weeks V and VI show the efficiency of copper sulphate in killing *B. coli*. Although the 1 cubic centimeter portions showed no difference, 10 cubic centimeter inoculations showed (Table XIII) that the average of 2.33 positive *B. coli* tests per week, obtained when 1 part of copper sulphate per million parts of water was used, was reduced to 0.66 times when 2 parts were applied. During week VII, when no disinfectant was used, the *B. coli* count again increased, but during the following week it was reduced by the daily application of 0.5 part of chlorine. Continuing the same application for another week (Table IX), the increase in the *B. coli* count, as well as that of other bacteria, showed that it was a disadvantage to use the water in a tank longer than one week. When fresh water was again used, the daily addition of antiformin, equivalent to one-seventh part of available chlorine per million parts of water (Table X), reduced the *B. coli* count to 2, while the daily addition of 0.5 part of chlorine (as chloride of lime) brought it down to zero. In an attempt further to reduce the amount of chloride of lime, 0.25 part of available chlorine was added daily during week XI. However, *B. coli* again made its appearance and the total bacterial counts gradually increased, showing that this amount of disinfectant was insufficient.

The total bacterial counts, with daily variations and the effect of some of the disinfectants, are shown in fig. 1, where the Roman numerals refer to the tables from which the averages are taken. The 37°C. counts are higher than the 25°C. counts, and in both cases the curve obtained when no disinfectant was added (VII) is highest. When 0.5 part of chlorine per million parts of water was used once a week, the bacteria were kept down somewhat, but at the end of the week they were nearly as numerous as in the former case. However, III (1 part of chlorine weekly) shows a considerable bacterial reduction at the end of the week. This reduction is more pronounced in the case of IV (2 parts of chlorine weekly). The most efficient disinfection was obtained when 0.5 part of chlorine (from chloride of lime) was added daily. This is represented by the dotted line VIII. In spite of the fact that curves based upon bacterial count often show surprisingly great jumps, which cannot be accounted for, these curves illustrate the conditions very

well and would probably show greater uniformity if a larger number of tests had been made or a greater number of pools could have been examined.

By comparing the bacterial counts of pools I and III in Tables VIII, IX, and XI, it is noticeable, especially in the last table, that the calcium hypochlorite seemed to have less disinfecting power in pool III than in pool I. This was true for both *B. coli* and total counts. In seeking a possible explanation, we find that it was not due to the difference in the number of bathers, as the daily average, during these weeks, of the people who used pool I was 19, whereas it was only 17 for pool III. The probable reason seems to be that the latter pool was open at the sides, admitting an abundance of light which caused a deterioration of the disinfectant. That light hastens the decomposition of the hypochlorite has already been mentioned.

During weeks VIII and IX the same water was used with the daily addition of 0.5 part of available chlorine from calcium hypochlorite per million parts of water. However, on the intervening Sunday no disinfectant was added. The effect was readily observable by the sudden increase of the bacterial count on the following day.

RELATION OF TURBIDITY AND BACTERIAL CONTENT

As the strength of a bacterial emulsion is often judged from its turbidity, it was thought that possibly some relation might exist between the turbidity of a swimming-pool water and its bacterial content. However, in this case, besides the physical suspension of bacteria, new chemical compounds are formed by the addition of disinfectants. Table XIV is presented to show how these factors compare.

TABLE XIV.—Comparison of turbidity and bacterial content of water in swimming pools.

[Average of 25° and 37° C. count.]

POOL I.

Age of water in days.	VII. No disinfectant.		V. One part per million copper sulphate.		VI. Two parts per million copper sulphate.		IV. Two parts per million chlorine from calcium hypochlorite.	
	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.
1	13.5	280	11.5	45	12.8	550		3,500
2	8.5	9,300	10.5	1,850	13	23,000	13	550
3		14,000	10.7	45,500	12	195,000	15	6
4	8.4	270,000	10.5	230,000	13.5	2,300,000	10	11,500
5	8.4	90,000	10.5	450,000	12.4	5,700,000	10.5	1,850,000
6	8.8	550,000	9.7	165,000		17,500,000	13.5	175,000

TABLE XIV.—Comparison of turbidity and bacterial content of water in swimming pools—Continued.

## POOL III.

Age of water in days.	VII. No disinfectant.		V. One part per million copper sulphate.		VI. Two parts per million copper sulphate.		IV. Two parts per million chlorine from calcium hypochlorite.	
	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.
1.....			11.7	26,700		125,000	16	12,500
2.....			11.3	55,200	20	150,000	10.1	140
3.....	15.5	24,000	11.3	100,500	12	35,000	10.1	7
4.....	14.5	70,000	9.7	115,000		4,650	12.5	50,000
5.....	15	145,000	10.5	55,000	14	3,500	12.9	123,500
6.....	15.5	1,220,000			13	3,800,000	10.5	6,500

25°C. on agar.

37°C. on agar.

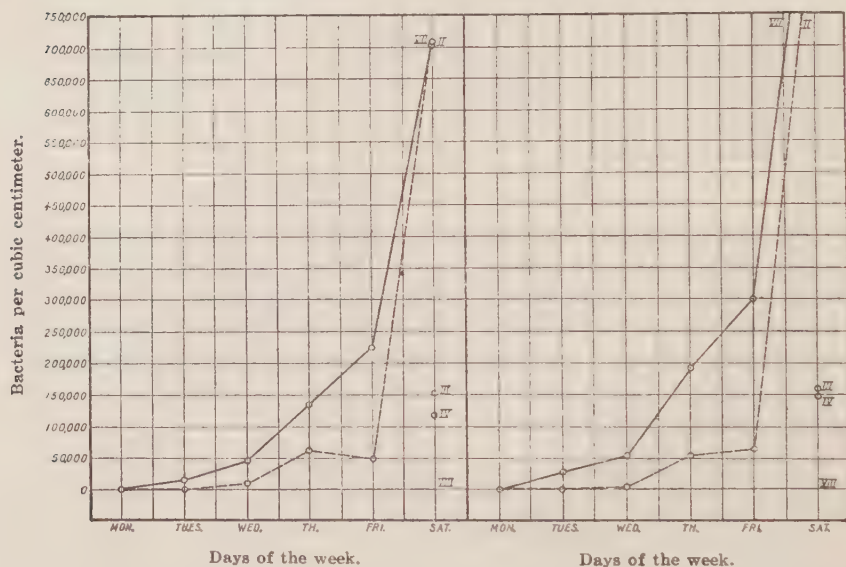


FIG. 1. Average daily counts of bacterial growth on agar at 25°C. and 37°C. The solid line was plotted from the data in Table VII; the dash line, from the data in Table II; and the dot line, from Table VIII. Only the respective end points are plotted for the curves resulting from the data in Tables III and IV.

As was anticipated, the addition of the disinfectants caused in pool I an increase in turbidity, further increased with larger amounts of disinfectant. Copper sulphate produced a greater turbidity than calcium hypochlorite. Pool III showed the same relations except that the recorded turbidity of the water to which no disinfectant was added was usually high. While in general an increased turbidity was associated with a decreased



bacterial content, no uniform relation between the two could be established.

#### SUMMARY AND CONCLUSIONS

Experiments have been made on three pools of 200 to 225 cubic meters' capacity, supplied with city water, which is a chlorinated river water with a temperature of about 28°C. The one hundred eighty-nine samples of 50 cubic centimeters of water were obtained about 1 meter below the surface and plated about forty minutes later on agar. After incubating at 25° and 37°C. for twenty-four hours, counts were made, and the positive presumptive tests for *B. coli* were further tested by plating.

Congo red lactose agar gave good results, but Endo medium seemed preferable for the confirmatory tests for *B. coli*. In the fermentation tubes lactose bile without peptone did not give as good results as lactose neutral red bouillon or lactose bouillon. One and 10 cubic centimeters of the water were inoculated, the latter into 30 cubic centimeters of the medium. *Bacillus coli* was found in the swimming pools to be usually not much more abundant than is accepted as permissible in drinking water. It was reduced most effectively by adding once a week 2 parts of copper sulphate per million parts of water. One week the daily addition of 0.5 part chlorine as calcium hypochlorite per million parts of water gave better results, while during another week the results were not as good as when the copper sulphate was used.

Copper sulphate produced a greater turbidity than calcium hypochlorite. An increase of disinfectant caused an increase in turbidity. Conversely, from the condition we find in a suspension of bacteria in water, due to the addition of a disinfectant, the number of bacteria often varied inversely as the turbidity, but not invariably. Consequently the determination of the turbidity would not be a practical test for determining the pollution in these pools. This can best be shown by regular tests for *B. coli* and the number of other bacteria present.

When a swimming pool is merely emptied and refilled, the bacterial count of the fresh water is much higher than when the emptied pool is thoroughly cleaned before refilling.

An increased amount of a given disinfectant invariably reduced the average numbers of *B. coli* and the total bacterial counts, as is graphically shown in fig. 1. This curve also shows the relation of the counts at 37°C. and those at 25°C., the former amounting to 116 per cent of the latter.

The general results of the experiments, as far as the bacterial count is concerned, may be summarized as follows:

1. When no disinfectant was added, there was a steady increase in the number of bacteria in the swimming pools. This increase was exceedingly high on the last day of the week the water was used.
2. When the disinfectants were used, the bacterial curve was lowered and often became more irregular.
  - a. Copper sulphate applied at the beginning of the week permitted an increase throughout the week, but sometimes a slight reduction occurred.
  - b. Calcium hypochlorite applied once a week showed good effects at first, but permitted too large an increase of bacteria toward the end of the week. A daily addition amounting to 0.25 part of available chlorine per million parts of water permitted a considerable increase of bacteria, while 0.5 part gave the best results.

When the comparative costs and disinfecting power are considered, antiformin and copper sulphate are not as valuable as calcium hypochlorite. The latter, when added daily in amounts equivalent to 0.5 part chlorine per million parts water, is recommended for the disinfection of these swimming pools, as it is the best and most economical means of keeping the number of intestinal and other bacteria within safe limits throughout the week in which the same water is used.

## ILLUSTRATION

### TEXT FIGURE

FIG. 1. Chart, showing average daily counts of bacterial growth on agar at 25°C. and 37°C. The solid line was plotted from the data in Table VII; the dash line, from the data in Table II; and the dot line, from Table VIII. Only the respective end points are plotted for the curves resulting from the data in Tables III and IV.





## WASSERMANN REACTION WITH GLYCERINATED HUMAN SERUM<sup>1</sup>

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In a previous report<sup>2</sup> I showed that glycerin is a suitable preservative for human serum intended for the Wassermann reaction. It prevents bacterial growth and does not materially influence the test. The sera previously reported on were studied for a short time only and were tested at weekly intervals; this report deals with sera tested monthly for a period of three months.

*Method.*—The Wassermann method with human hæmolytic system was used exclusively in this investigation.

*Complement.*—As complement serum the pooled sera of three guinea pigs were used in quantities of 0.1, 0.05, and 0.025 cubic centimeter.

*Antigen.*—The antigen was alcoholic extract of human-heart muscle, and was used in quantities of about one fourth of the anticomplementary dose for 1.25 unit of hæmolytic amboceptor with 0.05 cubic centimeter of complement serum. This may be done provided it is not anticomplementary in the antigen control, or an allowance is made when the results are read. If one fourth of the anticomplementary dose is anticomplementary in the antigen control, I prefer to use the largest quantity of antigen that does not show any anticomplementary property in the antigen control. The control should never be omitted. Throughout this investigation 1 cubic centimeter of alcoholic extract was diluted with 29 cubic centimeters of physiologic salt solution, and each tube received 0.5 cubic centimeter of the diluted antigen.

*Hæmolytic amboceptor.*—The serum of rabbits that had been immunized against washed human-blood corpuscles was used in doses of from 1 to 2 units per test tube. The term unit was

<sup>1</sup> Submitted for publication March 27, 1916.

<sup>2</sup> *This Journal, Sec. B* (1916), 11, 1.

applied to the smallest quantity of hæmolytic serum which with 0.05 cubic centimeter of complement dissolved the test dose of sensitized corpuscles in one hour. The mixture of hæmolytic amboceptor and corpuscles was allowed to stand at room temperature for thirty minutes before the complement was added. After the complement had been added, the tubes were placed in the incubator at 37°C. for one hour.

*Corpuscles.*—Human corpuscles from nonsyphilitic persons were well washed and were used in doses of 0.5 cubic centimeter of a 4 per cent suspension in physiologic salt solution. The corpuscles were sensitized for thirty minutes before they were added to the serum-complement-antigen mixture.

*Technique.*—The technique used in this investigation was identical with that described in the previous report. Before testing, the human sera were heated to about 55.5°C. (the temperature varied from 55.2°C. to 55.7°C.) for thirty minutes. Six-tenths cubic centimeter of the inactivated serum or 1.2 cubic centimeters of equal parts of human serum and chemically pure glycerin were diluted to 3 cubic centimeters with physiologic salt solution. Six test tubes, 1, 2, and 3 as antigen tubes and 1', 2', and 3' as control tubes, were used in each test. Each tube received 0.5 cubic centimeter of diluted serum. I preferred to use the constant quantity of serum in order to have the anti-complementary property uniform in all tubes. Each of the first pair of tubes, tubes 1 and 1', received 0.5 cubic centimeter of 1:5 dilution of complement serum; each tube of the second pair, tubes 2 and 2', received 0.5 cubic centimeter of 1:10 dilution of complement serum; and each of the third pair of tubes, tubes 3 and 3', received 0.5 cubic centimeter of 1:20 dilution of complement serum. Each of the antigen tubes, tubes 1, 2, and 3, received 0.5 cubic centimeter of 1:30 dilution of alcoholic extract (antigen); and the control tubes, tubes 1', 2', and 3', received 0.5 cubic centimeter of physiologic salt solution each. These mixtures were placed in the incubator at 37°C. for one hour. After having been in the incubator one hour, each tube received 1 cubic centimeter of sensitized corpuscles, representing 0.5 cubic centimeter of 4 per cent suspension of washed corpuscles and 1 unit of amboceptor diluted to 0.5 cubic centimeter with physiologic salt solution. After shaking, the tubes were placed in the incubator at 37°C. for one hour; during this hour and during the thirty minutes while the corpuscles were being sensitized, the mixtures were repeatedly shaken to prevent the

corpuscles from settling to the bottom of the container. After having been in the incubator for one hour, the tubes were allowed to stand at room temperature for two hours, after which the first reading was taken. After the first reading the tubes were put into the refrigerator, and the final results were read on the following morning.

*Antigen control.*—Six test tubes were used as in conducting the test, but the human serum was omitted, and the volume was made up with physiologic salt solution. If there was no anticomplementary action in the antigen control, the dose of antigen was considered suitable. If there was anticomplementary action, the dose of antigen was decreased until there was no anticomplementary action.

#### TEST 1

Specimens 4424, 4425, 4426, 4427, and 4428 were secured November 23, 1915. The sera were drawn off the clots the next day, and each serum was divided into two portions, A and B. Unglycerinated, portion A was tested November 24. Portion B, unheated, was mixed with an equal volume of sterilized, chemically pure glycerin and was kept at room temperature in a cork-stoppered test tube.

Specimens 4429, 4430, 4432, and 4433 were secured November 24, 1915. The sera were drawn off the clots the next day, and each serum was divided into two portions, A and B. Portion A, unglycerinated, was tested November 25. Unheated, portion B was mixed with an equal volume of sterilized, chemically pure glycerin and was kept at room temperature in a cork-stoppered test tube.

Specimen 4434 was secured November 26, 1915. The serum was drawn off the clot the next day and was divided into two portions, A and B. Unglycerinated; portion A was tested November 27. Unheated, portion B was mixed with an equal volume of sterilized, chemically pure glycerin and was kept at room temperature in a cork-stoppered test tube.

Portion B of each of the above sera was tested December 26, 1915, January 30, 1916, and February 22, 1916. Immediately before testing, the glycerinated serum (1.2 cubic centimeters) necessary for the test was heated to about 55.5°C. for thirty minutes.

All sera were tested bacteriologically.

TABLE I.—*Wassermann reaction with glycerinated human sera, heated immediately before testing.*

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube—						Result.
							1	2	3	1'	2'	3'	
4424	1915. Nov. 23	A	1915. Nov. 24	1915. Nov. 24	1.0	{ 1 0 0 0 + ± 0 2 tr 0 0 + + 0							Strongly positive. Do.
			1916. Dec. 26	1916. Dec. 26	1.25	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Jan. 30	1916. Jan. 30	1.25	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Feb. 22	1916. Feb. 22	1.5	{ 1 + 0 0 + + + 2 + 0 0 + + +							Do. Do.
		B	1915. Nov. 24	1915. Nov. 24	1.0	{ 1 + tr 0 + + 0 2 + ± 0 + + tr							Moderately positive. Do.
			1916. Dec. 26	1916. Dec. 26	1.25	{ 1 + 0 0 + ± 0 2 + 0 0 + ± 0							Do. Do.
			1916. Jan. 30	1916. Jan. 30	1.25	{ 1 + 0 0 + ± 0 2 + 0 0 + ± 0							Do. Do.
			1916. Feb. 22	1916. Feb. 22	1.5	{ 1 + + 0 + + ± 2 + + 0 + + ±							Do. Do.
4426	do	A	1915. Nov. 24	1915. Nov. 24	1.0	{ 1 tr 0 0 + ± 0 2 tr 0 0 + ± 0							Strongly positive. Do.
			1916. Dec. 26	1916. Dec. 26	1.25	{ 1 0 0 0 + tr 0 2 0 0 0 + tr 0							Do. Do.
			1916. Jan. 30	1916. Jan. 30	1.25	{ 1 ± 0 0 + ± 0 2 ± 0 0 + ± 0							Do. Do.
			1916. Feb. 22	1916. Feb. 22	1.5	{ 1 + tr 0 + + ± 2 + tr 0 + + ±							Do. Do.
		B	1915. Nov. 24	1915. Nov. 24	1.0	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Dec. 26	1916. Dec. 26	1.25	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Jan. 30	1916. Jan. 30	1.25	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Feb. 22	1916. Feb. 22	1.5	{ 1 + 0 0 + + ± 2 + 0 0 + + ±							Do. Do.
4427	do	A	1915. Nov. 24	1915. Nov. 24	1.0	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Dec. 26	1916. Dec. 26	1.25	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Jan. 30	1916. Jan. 30	1.25	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Feb. 22	1916. Feb. 22	1.5	{ 1 + 0 0 + + ± 2 + 0 0 + + ±							Do. Do.
		B	1915. Nov. 24	1915. Nov. 24	1.0	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Dec. 26	1916. Dec. 26	1.25	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Jan. 30	1916. Jan. 30	1.25	{ 1 tr 0 0 + + 0 2 tr 0 0 + + 0							Do. Do.
			1916. Feb. 22	1916. Feb. 22	1.5	{ 1 + 0 0 + + ± 2 + 0 0 + + ±							Do. Do.
4428	do	A	1915. Nov. 24	1915. Nov. 24	1.0	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Dec. 26	1916. Dec. 26	1.25	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Jan. 30	1916. Jan. 30	1.25	{ 1 tr 0 0 + + 0 2 tr 0 0 + + 0							Do. Do.
			1916. Feb. 22	1916. Feb. 22	1.5	{ 1 + 0 0 + + ± 2 + 0 0 + + ±							Do. Do.
		B	1915. Nov. 24	1915. Nov. 24	1.0	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Dec. 26	1916. Dec. 26	1.25	{ 1 0 0 0 + ± 0 2 0 0 0 + ± 0							Do. Do.
			1916. Jan. 30	1916. Jan. 30	1.25	{ 1 tr 0 0 + + 0 2 tr 0 0 + + 0							Do. Do.
			1916. Feb. 22	1916. Feb. 22	1.5	{ 1 + 0 0 + + ± 2 + 0 0 + + ±							Do. Do.



TABLE I.—Wassermann reaction with glycerinated human sera, heated immediately before testing—Continued.

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube—						Result.
							1	2	3	1'	2'	3'	
4429	Nov. 23	A	1915. Nov. 24	1915. Nov. 24	1.0	{ 1	0	0	0	+	+	0	Do.
						{ 2	0	0	0	+	+	0	Do.
		B	1915. Dec. 26	1915. Dec. 26	1.25	{ 1	0	0	0	+	±	0	Do.
						{ 2	0	0	0	+	±	0	Do.
		B	1916. Jan. 30	1916. Jan. 30	1.25	{ 1	0	0	0	+	±	0	Do.
						{ 2	0	0	0	+	±	0	Do.
		B	1916. Feb. 22	1916. Feb. 22	1.5	{ 1	+	0	0	+	+	tr	Do.
						{ 2	+	0	0	+	+	tr	Do.
4430	Nov. 24	A	1915. Nov. 25	1915. Nov. 25	1.0	{ 1	+	±	0	+	+	0	Weakly positive.
						{ 2	+	±	0	+	+	0	Do.
		B	1915. Dec. 26	1915. Dec. 26	1.25	{ 1	+	tr	0	+	±	0	Do.
						{ 2	+	tr	0	+	±	0	Do.
		B	1916. Jan. 30	1916. Jan. 30	1.25	{ 1	+	tr	0	+	±	0	Moderately positive.
						{ 2	+	tr	0	+	±	0	Do.
		B	1916. Feb. 22	1916. Feb. 22	1.5	{ 1	+	+	tr	+	+	±	Do.
						{ 2	+	+	tr	+	+	±	Do.
4432	do	A	1915. Nov. 25	1915. Nov. 25	1.0	{ 1	0	0	0	+	±	0	Strongly positive.
						{ 2	0	0	0	+	±	0	Do.
		B	1915. Dec. 26	1915. Dec. 26	1.25	{ 1	0	0	0	+	±	0	Do.
						{ 2	0	0	0	+	±	0	Do.
		B	1916. Jan. 30	1916. Jan. 30	1.25	{ 1	0	0	0	+	±	0	Do.
						{ 2	0	0	0	+	±	0	Do.
		B	1916. Feb. 22	1916. Feb. 22	1.5	{ 1	+	0	0	+	+	tr	Do.
						{ 2	+	0	0	+	+	tr	Do.
4433	do	A	1915. Nov. 25	1915. Nov. 25	1.0	{ 1	+	±	0	+	+	0	Weakly positive.
						{ 2	+	±	0	+	+	0	Do.
		B	1915. Dec. 26	1915. Dec. 26	1.25	{ 1	±	0	0	+	tr	0	Moderately positive.
						{ 2	±	0	0	+	tr	0	Do.
		B	1916. Jan. 30	1916. Jan. 30	1.25	{ 1	+	0	0	+	±	0	Do.
						{ 2	+	0	0	+	±	0	Do.
		B	1916. Feb. 22	1916. Feb. 22	1.5	{ 1	+	+	0	+	+	tr	Weakly positive.
						{ 2	+	+	0	+	+	tr	Do.
4434	do	A	1915. Nov. 25	1915. Nov. 25	1.0	{ 1	tr	0	0	+	±	0	Strongly positive.
						{ 2	tr	0	0	+	±	0	Do.
		B	1915. Dec. 26	1915. Dec. 26	1.25	{ 1	tr	0	0	+	±	0	Do.
						{ 2	tr	0	0	+	±	0	Do.
		B	1916. Jan. 30	1916. Jan. 30	1.25	{ 1	0	0	0	+	±	0	Do.
						{ 2	0	0	0	+	±	0	Do.
		B	1916. Feb. 22	1916. Feb. 22	1.5	{ 1	+	0	0	+	+	±	Do.
						{ 2	+	0	0	+	+	±	Do.

Explanation: +, complete hæmolysis; ±, hæmolysis between 50 per cent and 100 per cent; tr (trace), hæmolysis less than 50 per cent; 0, no hæmolysis.

Table I shows that with these ten sera, namely, Nos. 4424, 4425, 4426, 4427, 4428, 4429, 4430, 4432, 4433, and 4434, glycerin did not influence the Wassermann reaction. Sera 4430 and 4433 gave weakly positive results at the first test and moderately positive results at subsequent tests. With the other sera subsequent tests gave results practically identical with the results of the first tests. After having been kept at room temperature for three months, these sera were bacteriologically sterile, and after having been heated to about 55.5°C. for thirty minutes, they were but slightly anticomplementary. There was little or no difference between the two readings.

#### TEST 2

Specimens 4435, 4436, 4437, 4438, and 4439 were secured November 26, 1915. The next day the sera were drawn off the clots, and each serum was divided into two portions, A and B. Without having been mixed with glycerin, portion A of each serum was tested November 27. Portion B of each serum was heated to about 55.5°C. for thirty minutes November 27, was mixed with an equal volume of sterilized, chemically pure glycerin, and was kept at room temperature in a cork-stoppered test tube to be tested later.

Specimens 4440, 4441, 4442, 4443, and 4444 were secured November 27. The sera were drawn off the clots the next day. Each serum was divided into two portions, A and B. Unglycerinated, portion A was tested November 28. On the same date portion B of each serum was heated to about 55.5°C. for thirty minutes, was mixed with an equal volume of sterilized, chemically pure glycerin, and was kept at room temperature in a cork-stoppered test tube to be tested later.

Without having been reheated, portion B of each serum was tested December 27, 1915, January 30, 1916, and February 27, 1916.

February 27, 1916, each serum was tested bacteriologically.

Table II shows that with the ten sera used in test 2 the glycerin did not noticeably influence the Wassermann reaction. After having been kept at room temperature for three months, the results obtained with the test were practically identical with the results obtained before the sera had been mixed with glycerin. The sera had not become anticomplementary and were free from bacterial growth. There was little or no difference between the reading taken three hours after the corpuscles had been added and that taken eighteen hours after the corpuscles had been added.

TABLE II.—Wassermann reaction with human sera, heated before having been mixed with the glycerin.

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Ambocep- tor unit.	Reading.	Tube—						Result.
							1	2	3	1'	2'	3'	
4435	1915. Nov. 26	A	1915. Nov. 27	1915. Nov. 27	1.0	1	tr	0	0	+	+	0	Strongly positive.
						2	tr	0	0	+	+	0	Do.
		B	do	1915. Dec. 27	1.25	1	±	0	0	+	+	tr	Do.
						2	±	0	0	+	+	tr	Do.
		B	do	1916. Jan. 30	1.25	1	+	0	0	+	+	±	Do.
						2	+	0	0	+	+	±	Do.
		B	do	1916. Feb. 27	1.25	1	tr	0	0	+	tr	0	Do.
						2	tr	0	0	+	tr	0	Do.
4436	do	A	do	1915. Nov. 27	1.0	1	+	tr	0	+	±	0	Weakly positive.
						2	+	±	0	+	+	0	Do.
		B	do	1915. Dec. 27	1.25	1	+	±	0	+	+	tr	Moderately positive.
						2	+	±	0	+	+	tr	Do.
		B	do	1916. Jan. 30	1.25	1	+	+	0	+	+	tr	Weakly positive.
						2	+	+	0	+	+	tr	Do.
		B	do	1916. Feb. 27	1.25	1	±	0	0	+	tr	0	Moderately positive.
						2	±	0	0	+	tr	0	Do.
4437	do	A	do	1915. Nov. 27	1.0	1	0	0	0	+	±	0	Strongly positive.
						2	0	0	0	+	±	0	Do.
		B	do	1915. Dec. 27	1.25	1	tr	0	0	+	+	tr	Do.
						2	tr	0	0	+	+	tr	Do.
		B	do	1916. Jan. 30	1.25	1	±	0	0	+	+	±	Do.
						2	±	0	0	+	+	±	Do.
		B	do	1916. Feb. 27	1.25	1	tr	0	0	+	±	0	Do.
						2	tr	0	0	+	±	0	Do.
4438	do	A	do	1915. Nov. 27	1.0	1	+	0	0	+	+	tr	Do.
						2	+	0	0	+	+	tr	Do.
		B	do	1915. Dec. 27	1.25	1	±	0	0	+	+	tr	Do.
						2	±	0	0	+	+	tr	Do.
		B	do	1916. Jan. 30	1.25	1	+	0	0	+	+	±	Do.
						2	+	0	0	+	+	±	Do.
		B	do	1916. Feb. 27	1.25	1	±	0	0	+	+	0	Do.
						2	±	0	0	+	+	0	Do.
4439	do	A	do	1915. Nov. 27	1.0	1	+	±	0	+	+	0	Weakly positive.
						2	+	±	0	+	+	0	Do.
		B	do	1915. Dec. 27	1.25	1	+	+	0	+	+	±	Moderately positive.
						2	+	+	0	+	+	±	Do.
		B	do	1916. Jan. 30	1.25	1	+	+	0	+	+	±	Do.
						2	+	+	0	+	+	±	Do.
		B	do	do	1.25	1	+	tr	0	+	+	0	Do.
						2	+	tr	0	+	+	0	Do.

TABLE II.—*Wassermann reaction with human sera, heated before having been mixed with the glycerin—Continued.*

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube—						Result.
							1	2	3	1'	2'	3'	
4440	Nov. 27	A	1915. Nov. 28	1915. Nov. 28	1.0	1	tr	0	0	+	+	tr	Strongly positive.
						2	tr	0	0	+	+	tr	Do.
		B	do	Dec. 27	1.25	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
		B	do	1916. Jan. 30	1.25	1	0	0	0	+	+	tr	Do.
						2	0	0	0	+	+	tr	Do.
4441	do	B	do	Feb. 27	1.25	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
		A	do	1915. Nov. 28	1.0	1	0	0	0	+	+	tr	Do.
						2	0	0	0	+	+	tr	Do.
		B	do	Dec. 27	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
4442	do	B	do	1916. Jan. 30	1.25	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
		B	do	Feb. 27	1.25	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
		A	do	1915. Nov. 28	1.0	1	+	0	0	+	+	tr	Do.
						2	+	0	0	+	+	tr	Do.
4443	do	B	do	Dec. 27	1.25	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
		B	do	1916. Jan. 30	1.25	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
		B	do	Feb. 27	1.25	1	0	0	0	+	+	tr	Do.
						2	0	0	0	+	+	tr	Do.
4444	do	A	do	1915. Nov. 28	1.0	1	tr	0	0	+	+	0	Do.
						2	tr	0	0	+	+	0	Do.
		B	do	Dec. 27	1.25	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
		B	do	1916. Jan. 30	1.25	1	0	0	0	+	+	tr	Do.
						2	0	0	0	+	+	tr	Do.
4444	do	B	do	Feb. 27	1.25	1	tr	0	0	+	+	tr	Do.
						2	tr	0	0	+	+	tr	Do.
		A	do	1915. Nov. 28	1.0	1	+	tr	0	+	±	0	Weakly positive.
						2	+	tr	0	+	±	0	Do.
		B	do	Dec. 27	1.25	1	+	tr	0	+	±	0	Do.
						2	+	tr	0	+	±	0	Do.
4444	do	B	do	1916. Jan. 30	1.25	1	+	+	0	+	+	tr	Do.
						2	+	+	0	+	+	tr	Do.
		B	do	Feb. 27	1.25	1	+	+	0	+	+	tr	Do.
						2	+	+	0	+	+	tr	Do.
		B	do	Feb. 27	1.25	1	+	+	0	+	+	tr	Do.
						2	+	+	0	+	+	tr	Do.



## TEST 3

November 30, 1915, specimens 4446, 4447, 4448, 4449, and 4450 were secured. The sera were drawn off the clots the next day. Each serum was divided into two portions, A and B. Unglycerinated, portion A was tested December 1. Portion B was heated to 55.5°C. for thirty minutes, was mixed with an equal volume of sterilized, chemically pure glycerin, was placed in the cold storage at about 7°C. in a cork-stoppered test tube, and was tested at intervals of about a month. About three months after it was mixed with glycerin it was examined bacteriologically.

Table III shows that with sera 4446, 4447, 4448, 4449, and 4450 glycerin did not influence the Wassermann reaction during the period of three months. The results obtained at the end of one month, at the end of two months, and at the end of three months were practically identical with the results obtained before glycerin had been added to the sera. The sera having been heated on December 1, 1915, before they had been mixed with glycerin, did not become anticomplementary in three months, and all remained free from bacterial growth.

## TEST 4

Specimens 4452, 4453, 4454, 4455, and 4456 were secured December 1, 1915. The next day the sera were drawn off the clots. Each serum was divided into two portions, A and B. Unglycerinated, portion A was tested December 2. Portion B of each serum was mixed with an equal volume of sterilized, chemically pure glycerin, was placed in the cold storage at a temperature of about 7°C., and was tested at intervals of about a month. The necessary quantity of serum was heated immediately before the test.

On March 4, 1916, a bacteriologic test was made of each serum.

TABLE III.—Wassermann reaction with glycerinated human sera, heated before having been mixed with glycerin; kept in cold storage.

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube—						Result.
							1	2	3	1'	2'	3'	
4446	1915. Nov. 30	A	1915. Dec. 1	1915. Dec. 1	1.0	1	+	0	0	+	+	tr	Strongly positive.
						2	+	0	0	+	+	tr	Do.
		B	do	1916. Jan. 2	1.25	1	tr	0	0	+	+	0	Do.
						2	tr	0	0	+	+	0	Do.
		B	do	Feb. 6	1.5	1	+	0	0	+	+	+	Do.
						2	+	0	0	+	+	+	Do.
		B	do	Mar. 4	1.25	1	tr	0	0	+	±	0	Do.
						2	tr	0	0	+	±	0	Do.
4447	do	A	do	1915. Dec. 1	1.0	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
		B	do	1916. Jan. 2	1.25	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
		B	do	Feb. 6	1.5	1	0	0	0	+	+	+	Do.
						2	0	0	0	+	+	+	Do.
		B	do	Mar. 4	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
4448	do	A	do	1915. Dec. 1	1.0	1	+	tr	0	+	+	tr	Do.
						2	+	tr	0	+	+	tr	Do.
		B	do	1916. Jan. 2	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
		B	do	Feb. 6	1.5	1	+	0	0	+	+	+	Do.
						2	+	0	0	+	+	+	Do.
		B	do	Mar. 4	1.25	1	tr	0	0	+	±	0	Do.
						2	tr	0	0	+	±	0	Do.
4449	do	A	do	1915. Dec. 1	1.0	1	0	0	0	+	+	tr	Do.
						2	0	0	0	+	+	tr	Do.
		B	do	1916. Jan. 2	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
		B	do	Feb. 6	1.5	1	0	0	0	+	+	+	Do.
						2	0	0	0	+	+	+	Do.
		B	do	Mar. 4	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
4450	do	A	do	1915. Dec. 1	1.0	1	0	0	0	+	+	tr	Do.
						2	0	0	0	+	+	tr	Do.
		B	do	1916. Jan. 2	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
		B	do	Feb. 6	1.5	1	0	0	0	+	+	+	Do.
						2	0	0	0	+	+	+	Do.
		B	do	Mar. 4	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.

TABLE IV.—Wassermann reaction with glycerinated human sera kept in the cold storage, heated immediately before testing.

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Ambocep- tor unit.	Reading.	Tube—						Result.
							1	2	3	1'	2'	3'	
4452	Dec. 1	A	1915. Dec. 2	1915. Dec. 2	2.0	1	tr	0	0	+	+	tr	Strongly positive.
			1916. Jan. 2	1916. Jan. 2	1.25	2	tr	0	0	+	+	tr	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	+	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	+	0	0	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	+	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	+	0	0	Do.
		A	1915. Dec. 2	1915. Dec. 2	1.0	1	+	0	0	+	±	0	Moderately positive.
			1916. Jan. 2	1916. Jan. 2	1.25	2	+	0	0	+	±	0	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	±	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	±	0	0	Do.
4453	do	A	1915. Dec. 2	1915. Dec. 2	1.0	1	tr	0	0	+	+	tr	Strongly positive.
			1916. Jan. 2	1916. Jan. 2	1.25	2	tr	0	0	+	+	tr	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	+	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	+	0	0	Do.
		A	1915. Dec. 2	1915. Dec. 2	1.0	1	+	0	0	+	+	tr	Do.
			1916. Jan. 2	1916. Jan. 2	1.25	2	+	0	0	+	+	tr	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	±	0	0	Positive.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	±	0	0	Do.
		A	1915. Dec. 2	1915. Dec. 2	1.0	1	0	0	0	+	0	0	Strongly positive.
			1916. Jan. 2	1916. Jan. 2	1.25	2	0	0	0	+	0	0	Do.
4454	do	B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	+	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	+	0	0	Do.
		A	1915. Dec. 2	1915. Dec. 2	1.0	1	+	0	0	+	+	tr	Do.
			1916. Jan. 2	1916. Jan. 2	1.25	2	+	0	0	+	+	tr	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	±	0	0	Positive.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	±	0	0	Do.
		A	1915. Dec. 2	1915. Dec. 2	1.0	1	0	0	0	+	0	0	Strongly positive.
			1916. Jan. 2	1916. Jan. 2	1.25	2	0	0	0	+	0	0	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	+	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	+	0	0	Do.
4455	do	A	1915. Dec. 2	1915. Dec. 2	1.0	1	0	0	0	+	+	tr	Do.
			1916. Jan. 2	1916. Jan. 2	1.25	2	0	0	0	+	+	tr	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	+	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	+	0	0	Do.
		A	1915. Dec. 2	1915. Dec. 2	1.0	1	0	0	0	+	+	tr	Do.
			1916. Jan. 2	1916. Jan. 2	1.25	2	0	0	0	+	+	tr	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	+	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	+	0	0	Do.
		A	1915. Dec. 2	1915. Dec. 2	1.0	1	0	0	0	+	+	tr	Do.
			1916. Jan. 2	1916. Jan. 2	1.25	2	0	0	0	+	+	tr	Do.
4456	do	B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	+	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	+	0	0	Do.
		A	1915. Dec. 2	1915. Dec. 2	1.0	1	0	0	0	+	+	tr	Do.
			1916. Jan. 2	1916. Jan. 2	1.25	2	0	0	0	+	+	tr	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	+	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	+	0	0	Do.
		A	1915. Dec. 2	1915. Dec. 2	1.0	1	0	0	0	+	+	tr	Do.
			1916. Jan. 2	1916. Jan. 2	1.25	2	0	0	0	+	+	tr	Do.
		B	1915. Feb. 6	1915. Feb. 6	1.5	1	0	0	0	+	0	0	Do.
			1916. Mar. 4	1916. Mar. 4	2.0	2	0	0	0	+	0	0	Do.

Table IV shows that with sera 4452, 4453, 4454, 4455, and 4456 the glycerin did not noticeably influence the Wassermann reaction. All of these sera became strongly anticomplementary, far more so than did the sera that were kept at room temperature. All sera remained free from bacterial growth.

## TEST 5

Specimens 4535, 4536, 4537, 4538, and 4539 were secured December 27, 1915. The sera were drawn off the clots the next day. Each serum was divided into two portions, A and B. Unglycerinated, portion A was tested on December 28. Portion B of each serum was mixed with an equal volume of sterilized, chemically pure glycerin, was kept at room temperature in a cork-stoppered test tube, and was tested at intervals of a month. The necessary quantity of serum was heated to 55.5° C. immediately before testing. A bacteriologic test was made of each serum on March 18, 1916.

Table V shows that with sera 4535, 4536, 4537, 4538, and 4539 the glycerin did not noticeably influence the Wassermann reaction. Although the sera had not been heated, they did not become very strongly anticomplementary in about three months. The erratic results obtained on February 13, 1916, are not easily explained. All sera remained clear and free from bacterial growth.

## TEST 6

Specimens 4540, 4541, 4542, 4543, and 4544 were secured on December 27, 1915. December 28 the sera were drawn off the clots. Each serum was divided into two portions, A and B. Unglycerinated, portion A was tested December 28. Portion B was heated to about 55.5°C. for thirty minutes, was mixed with an equal volume of sterilized, chemically pure glycerin, was kept at room temperature in a cork-stoppered test tube, and was tested at intervals of about a month. On March 18, 1916, each serum was tested bacteriologically.



TABLE V.—Wassermann reaction with glycerinated human sera, kept at room temperature, heated immediately before testing.

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube—						Result.		
							1	2	3	1'	2'	3'			
4535	Dec. 27	A	1915. Dec. 28	1915. Dec. 28	1.0	{ 1 + + tr + + tr 2 + + tr + + tr							Negative. Do.		
			1916. Jan. 23	1916. Jan. 23	1.25	{ 1 + tr 0 + tr 0 2 + tr 0 + tr 0						Do. Do.			
		B	Feb. 13	Feb. 13	1.5	{ 1 + + + + + 2 + + + + +							None. Do.		
			Mar. 18	Mar. 18	1.5	{ 1 + ± 0 + ± 0 2 + ± 0 + ± 0							Negative. Do.		
		A	1915. Dec. 28	1915. Dec. 28	1.0	{ 1 + + tr + + tr 2 + + tr + + tr							Do. Do.		
			1916. Jan. 23	1916. Jan. 23	1.25	{ 1 + tr 0 + tr 0 2 + tr 0 + tr 0							Do. Do.		
		B	Feb. 13	Feb. 13	1.5	{ 1 + 0 0 + 0 0 2 + 0 0 + 0 0							Do. Do.		
			Mar. 18	Mar. 18	1.5	{ 1 + ± 0 + ± 0 2 + ± 0 + ± 0							Do. Do.		
		4537	do	A	1915. Dec. 28	1915. Dec. 28	1.0	{ 1 + + 0 + + 0 2 + + 0 + + 0							Do. Do.
					1916. Jan. 23	1916. Jan. 23	1.25	{ 1 + tr 0 + tr 0 2 + tr 0 + tr 0							Do. Do.
B	Feb. 13			Feb. 13	1.5	{ 1 + + + + + 2 + + + + +							None. Do.		
	Mar. 18			Mar. 18	1.5	{ 1 + + tr + + tr 2 + + tr + + tr							Negative. Do.		
A	1915. Dec. 28			1915. Dec. 28	1.0	{ 1 + + 0 + + 0 2 + + 0 + + 0							Do. Do.		
	1916. Jan. 23			1916. Jan. 23	1.25	{ 1 + tr 0 + tr 0 2 + tr 0 + tr 0							Do. Do.		
B	Feb. 13			Feb. 13	1.5	{ 1 + + + + + 2 + + + + +							None. Do.		
	Mar. 18			Mar. 18	1.5	{ 1 + + tr + + tr 2 + + tr + + tr							Negative. Do.		
4538	do			A	1915. Dec. 28	1915. Dec. 28	1.0	{ 1 + + 0 + + 0 2 + + 0 + + 0							Do. Do.
					1916. Jan. 23	1916. Jan. 23	1.25	{ 1 + tr 0 + tr 0 2 + tr 0 + tr 0							Do. Do.
		B	Feb. 13	Feb. 13	1.5	{ 1 + + + + + 2 + + + + +							None. Do.		
			Mar. 18	Mar. 18	1.5	{ 1 + + tr + + tr 2 + + tr + + tr							Negative. Do.		
		A	1915. Dec. 28	1915. Dec. 28	1.0	{ 1 + + 0 + + 0 2 + + 0 + + 0							Do. Do.		
			1916. Jan. 23	1916. Jan. 23	1.25	{ 1 + 0 0 + 0 0 2 + 0 0 + 0 0							Do. Do.		
		B	Feb. 13	Feb. 13	1.5	{ 1 + + 0 + + 0 2 + + 0 + + 0							Do. Do.		
			Mar. 18	Mar. 18	1.5	{ 1 + ± 0 + ± 0 2 + ± 0 + ± 0							Do. Do.		
		4539	do	A	1915. Dec. 28	1915. Dec. 28	1.0	{ 1 + + 0 + + 0 2 + + 0 + + 0							Do. Do.
					1916. Jan. 23	1916. Jan. 23	1.25	{ 1 + 0 0 + 0 0 2 + 0 0 + 0 0							Do. Do.
B	Feb. 13			Feb. 13	1.5	{ 1 + + 0 + + 0 2 + + 0 + + 0							Do. Do.		
	Mar. 18			Mar. 18	1.5	{ 1 + ± 0 + ± 0 2 + ± 0 + ± 0							Do. Do.		

TABLE VI.—Wassermann reaction with glycerinated human sera, heated before having been mixed with the glycerin, kept at room temperature.

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube—						Result.
							1	2	3	1'	2'	3'	
4540	Dec. 27	A	1915. Dec. 28	1915. Dec. 28	1.0	1	+	+	tr	+	+	tr	Negative.
						2	+	+	tr	+	+	tr	Do.
		B	do	1916. Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
						2	+	tr	0	+	tr	0	Do.
		B	do	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	do	Mar. 18	1.25	1	+	tr	0	+	tr	0	Negative.
						2	+	tr	0	+	tr	0	Do.
4541	do	A	do	1915. Dec. 28	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
		B	do	1916. Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
						2	+	tr	0	+	tr	0	Do.
		B	do	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	do	Mar. 18	1.25	1	+	tr	0	+	tr	0	Negative.
						2	+	tr	0	+	tr	0	Do.
4542	do	A	do	1915. Dec. 28	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
		B	do	1916. Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
						2	+	tr	0	+	tr	0	Do.
		B	do	Feb. 13	1.5	1	+	+	±	+	+	±	Do.
						2	+	+	±	+	+	±	Do.
		B	do	Mar. 18	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
4543	do	A	do	1915. Dec. 28	1.0	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	do	1916. Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
						2	+	tr	0	+	tr	0	Do.
		B	do	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	do	Mar. 18	1.25	1	+	±	0	+	±	0	Negative.
						2	+	±	0	+	±	0	Do.
4544	do	A	do	1915. Dec. 28	1.0	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	do	1916. Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
						2	+	tr	0	+	tr	0	Do.
		B	do	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	do	Mar. 18	1.25	1	+	±	0	+	±	0	Negative.
						2	+	±	0	+	±	0	Do.

Table VI shows the results obtained with sera 4540, 4541, 4542, 4543, and 4544. The glycerin did not noticeably influence the Wassermann reaction. The sera did not become anticomplementary. They remained clear and free from bacterial growth.

## TEST 7

On December 29, 1915, specimens 4546, 4547, 4548, 4549, and 4550 were secured. The sera were drawn off the clots December 30. Each serum was divided into two portions, A and B. Unglycerinated, portion A of each serum was tested December 30. Unheated, portion B of each serum was mixed with an equal volume of sterilized, chemically pure glycerin, was kept in a cork-stoppered test tube in the cold storage at a temperature of about 7°C., and was tested at intervals of about a month. The necessary quantity of serum was heated to 55.5°C. for thirty minutes immediately before testing.

Table VII shows that with sera 4546, 4547, 4548, 4549, and 4550 the glycerin did not influence the Wassermann reaction. The sera did not become very strongly anticomplementary in about three months, and all sera remained clear and free from bacterial growth. There was practically no difference between the first and second readings.

## TEST 8

Specimens 4551, 4552, 4553, 4554, and 4555 were secured December 29, 1915. The sera were drawn off the clots the next day. Each serum was divided into two portions, A and B. Unglycerinated, portion A of each serum was tested December 30. Portion B of each serum was heated to about 55.5°C. for thirty minutes, was mixed with an equal volume of sterilized, chemically pure glycerin, was kept in a cork-stoppered test tube in the cold storage at a temperature of about 7°C., and, without reheating, was tested at intervals of about a month. On March 25, 1916, each serum was tested for bacterial growth.

TABLE VII.—Wassermann reaction with glycerinated human sera, kept in the cold storage, heated immediately before testing.

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube—						Result.
							1	2	3	1'	2'	3'	
4546	Dec. 29	A	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Negative.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.
		B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	±	0	+	±	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	tr	0	+	tr	0	Do.
		B	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	±	0	+	±	0	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	±	0	+	±	0	Do.
		B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	0	0	+	0	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	0	0	+	0	0	Do.
		A	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.
4547	do	B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	0	0	+	0	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	0	0	+	0	0	Do.
		B	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.
		B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	0	0	+	0	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	0	0	+	0	0	Do.
		A	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.
		B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	0	0	+	0	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	0	0	+	0	0	Do.
4548	do	A	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.
		B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	0	0	+	0	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	0	0	+	0	0	Do.
		A	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.
		B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	0	0	+	0	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	0	0	+	0	0	Do.
		A	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.
4549	do	B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	0	0	+	0	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	0	0	+	0	0	Do.
		B	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.
		B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	0	0	+	0	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	0	0	+	0	0	Do.
		A	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.
		B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	0	0	+	0	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	0	0	+	0	0	Do.
4550	do	A	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.
		B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	±	0	+	±	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	±	0	+	±	0	Do.
		B	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	0	0	+	0	0	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	0	0	+	0	0	Do.
		B	1915. Feb. 20	1915. Feb. 20	1.25	{ 1	+	0	0	+	0	0	Do.
			1916. Mar. 25	1916. Mar. 25	1.5	{ 2	+	0	0	+	0	0	Do.
		A	1915. Dec. 30	1915. Dec. 30	1.0	{ 1	+	+	tr	+	+	tr	Do.
			1916. Jan. 23	1916. Jan. 23	1.25	{ 2	+	+	tr	+	+	tr	Do.



TABLE VIII.—Wassermann reaction with glycerinated human sera, heated before having been mixed with the glycerin, kept in the cold storage.

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Ambocep- tor unit.	Reading.	Tube—						Result.
							1	2	3	1'	2'	3'	
4551	1915. Dec. 29	A	1915. Dec. 30	1915. Dec. 30	1.0	1	+	+	tr	+	+	tr	Negative.
						2	+	+	tr	+	+	tr	Do.
		B	do	1916. Jan. 23	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
		B	do	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
	do	B	do	Mar. 25	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		A	do	1915. Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
		B	do	1916. Jan. 23	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
4552	do	B	do	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	do	Mar. 25	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		A	do	1915. Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
	do	B	do	1916. Jan. 23	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
		B	do	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	do	Mar. 25	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
4553	do	A	do	1915. Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
		B	do	1916. Jan. 23	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
		B	do	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
	do	B	do	Mar. 25	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
		A	do	1915. Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
		B	do	1916. Jan. 23	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
4554	do	B	do	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	do	Mar. 25	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		A	do	1915. Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
	do	B	do	1916. Jan. 23	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
		B	do	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	do	Mar. 25	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
4555	do	A	do	1915. Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
		B	do	1916. Jan. 23	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
		B	do	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
	do	B	do	Mar. 25	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		A	do	1915. Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
		B	do	1916. Jan. 23	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.

The results obtained are shown in Table VIII. With sera 4551, 4552, 4553, 4554, and 4555 the glycerin did not influence the Wassermann reaction. The sera had not become anticomplementary at the end of three months. There was practically no difference between the first and second readings. All sera remained clear and free from bacterial growth.

#### CONCLUSIONS

The following conclusions seem justified:

Chemically pure glycerin is an ideal preservative for human serum intended for the Wassermann reaction.

Serum mixed with an equal volume of sterilized, chemically pure glycerin remains clear and sterile for a long time.

In order to prevent the human serum from becoming anticomplementary, it must be heated to about 55°C. for thirty minutes before it is mixed with the glycerin.

Sera heated to 55.5°C. for thirty minutes and mixed with equal volumes of glycerin had not become anticomplementary at the end of three months.

Unheated human sera mixed with an equal volume of glycerin had not become unfit at the end of about three months. Such sera are moderately anticomplementary even after they have been heated to 55.5°C. for thirty minutes, but can be tested as well as fresh sera, provided more complement and more hæmolytic amboceptor are used. The anticomplementary property does not influence the result obtained with the test.

With sensitized human corpuscles hæmolysis is complete three hours after the complement has been added. If the tubes are to be compared with a hæmoglobin scale, they should be placed in the refrigerator for from twelve to twenty-four hours to allow the undissolved blood corpuscles to settle to the bottoms of the tubes.

I still have a few cubic centimeters of each of these sera and expect to report on them at intervals of a year.

## REVIEWS

Farmers | of | Forty Centuries | or | Permanent Agriculture in China, |  
Korea and Japan | by | F. H. King, D. Sc. | [6 lines] | Madison,  
Wis. | Mrs. F. H. King | 1911 | [1 line] | Cloth, pp. i-ix+1-441.  
Price, \$2.50 postpaid.

This volume is a valuable addition to the agricultural literature of the Far East.

The people of the Occident, save a few travelers who chanced to witness and study eastern agricultural activities, have little appreciation of the methods used by the people of the Orient in developing agricultural resources.

It has been commonly said that the nations of the West have much to teach their sisters in the East in the several lines of human activities, and vice versa, as far as they are concerned in the struggle for life. This being true, Professor King's book affords eloquent data and straightforward argument in support of what the Western people are lacking in agricultural development. This book is a valuable contribution to the universal endeavor toward improving the agriculture of the world as the backbone of wealth of mankind.

So far as the agriculture of these Islands is concerned, I unhesitatingly bespeak the acquisition of Professor King's book by all those who are truly devoted to farming. It will furnish them an opportune idea of the agricultural expansion of the inhabitants of the neighboring countries, thus enabling them to acquire lessons in connection with the betterment of the production of our staple crops, especially rice.

In my opinion the book should be translated into Spanish—the language commonly spoken among our farmers—in order to encourage its reading by the farmer.

ADN. HERNANDEZ.

A Practical Text-book | of | Infection, Immunity | and Specific Therapy | with  
special reference to immunologic technic | by | John A. Kolmer, M. D.,  
Dr. P. H., | [4 lines] | with an introduction by | Allen J. Smith, M. D.,  
Sc. D., LL. D. | [1 line] | with 143 original illustrations, 43 in colors  
| by Erwin F. Faber | [1 line] | Philadelphia and London | W. B.  
Saunders Company | 1915 | Cloth, pp. i-xi+1-899. Price, \$6.00 net;  
half morocco, \$7.50 net.

This volume serves a threefold purpose: It aims to give practitioners and students an account of our present knowledge of infection and immunity; to serve as a guide to the various immunologic methods for laboratory workers; and to outline a course for students.

The book is well illustrated with numerous half-tones and colored plates. It is divided into five parts: General Immunologic Technic; Principles of Infection; Principles of Immunity and Special Immunologic Technic; Applied Immunity in the Prophylaxis, Diagnosis, and Treatment of Disease—Specific Therapy; Experimental Infection and Immunity.

Part I gives clear and concise descriptions of laboratory apparatus and procedures which can easily be understood and followed by the reader.

Part II contains two chapters considering the various aspects of virulence and resistance, the various types of toxins, and the course of infection. Diphtheria toxin, tetanus toxin, snake venoms, aggrassin, ptomaines, and infection with animal parasites are discussed.

Part III deals with immunity and theories of immunity. The phagocytic theory of Metchnikoff and the side-chain theory of Ehrlich and different views regarding them are made clear to the reader. Bacterial vaccines, their preparation and use, anti-toxins, and bactericidal sera are described. The Abderhalden test constitutes one of the most important chapters in this part of the book. Each constituent is separately discussed, and in eight brief paragraphs the test is made so clear that any student understands it.

Various antibodies, such as agglutinins, precipitins, cytoly-sins, bacteriolysins, and hæmolysins are adequately discussed in separate chapters. The Wassermann reaction with its various modifications and controls is freely discussed and adequately illustrated. Perhaps never before has the Wassermann reaction been made so clear. Kolmer makes the student understand the test. Complement-fixation tests for other diseases are amply dealt with.

Part IV is wholly practical in its nature. The reader is given a survey of prophylactic vaccination, vaccine and serum therapy, and chemotherapy.

Part V consists of sixty exercises in experimental infection and immunity. The book is closed with a well-prepared index.

From a practical point of view the price of the book may seem high for the average student, especially as these subjects ad-



vance so rapidly that the student is continually compelled to consult current periodical literature. The book is worth it and can be highly recommended to students and practitioners of medicine.

E. H. RUEDIGER.

Fever | its Thermotaxis and | Metabolism | by | Isaac Ott, A. M., M. D. | [11 lines] | [seal] | Paul B. Hoeber | 67-69 E. 59th Street | New York | 1914 | Cloth, pp. 1-166. Price, \$1.50 net.

It is to be regretted that Professor Ott, who has contributed so much pioneer work on this subject, should appear as the author of the present booklet. This series of lectures seems to have been hastily written and very superficially edited. Professor Ott discusses particularly the heat centers, "thermotaxic nerves," heat dissipation and production, and metabolism on fever. The booklet is of little value either to the worker in the medical sciences or to the practitioner.

R. B. GIBSON.

Bacteriological Methods | in | Food and Drugs Laboratories | with an | introduction to micro-analytical methods | by | Albert Schneider, M. D., Ph. D. | [4 lines] | 87 illustrations | and 6 full page plates | Philadelphia | P. Blakiston's Son & Co. | 1012 Walnut Street | 1915 | Cloth, pp. i-viii+1-288. Price, \$2.50 net.

Within recent years increasing attention has been given in Food and Drugs Laboratories to bacteriological methods. The chemical methods formerly used almost exclusively did not furnish all the data demanded by modern sanitary requirements. Accordingly bacteriologists have been engaged to assist in the enforcement of the food and drug laws. However, their activities were handicapped by lack of definite information and standards on the subject, the fragments of knowledge which they could use on these subjects being widely scattered. To supply this growing and urgent need for a suitable book on bacteriological methods in food and drug laboratories, Doctor Schneider undertook the work here reviewed.

He prefaces the bacteriological methods proper by giving the essentials of microanalytical work, often required of bacteriologists in food and drug laboratories. Under bacteriological methods he considers the various means used to detect the number and kind of microorganisms which may be present in foods and drugs in a normal, adulterated, or spoiled condition and the significance of their presence. The methods and technique given are such as have been used and tested before. Their collection in one volume is a great convenience to a worker

in this line. The standardization of disinfectants usually treated in an unsatisfactory manner in most bacteriologies is fully considered. A bibliography would add to the usefulness of the book. However, it is the best book of its kind of which we know, it is clearly written, is well printed and illustrated, and contains much useful information.

CHAS. E. GABEL.

*Aids to Tropical Medicine* | by | Gilbert E. Brooke | M. A. Cantab., L. R. C. P. Edin., D. P. H., F. R. G. S. | Port Health Officer, Singapore | [7 lines] | second (seal) edition | London | Baillière, Tindall & Cox | 8, Henrietta Street, Covent Garden | 1915 | Cloth, pp. i-xii+1-230, Thirty-seven text figures. Price cloth,  $\frac{3}{4}$  net; paper, 3 net.

In this edition of "Aids to Tropical Medicine" by Brooke some new material has been added. The small size of the volume makes it convenient for use in the field. Like most books of this type omissions and errors have occurred in it. It is an open question whether books like this fill any real need in medical literature.

J. A. J.

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ON THE DEVELOPMENT OF TWINS AND OTHER POLYEMBRYOS  
WITH SPECIAL REFERENCE TO FOUR SETS OF DUCK  
TWINS<sup>1</sup>

By EDWARD S. RUTH

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University of the Philippines)*

THREE PLATES

For centuries there has been a great deal of speculation regarding the nature of the early development of twins and other polyembryos. In a few of the lower invertebrate forms some definite conclusions have been reached concerning polyembryony. Twins, triplets, quadruplets, and even more than four organisms have been produced experimentally from a single fertilized ovum.

Experimental production of twins and other polyembryos in mammals is impractical, and only a few theories have been established. First, twins may possibly develop from one ovum by a complete separation of the cells in the early stage of segmentation, possibly in the two- or four-cell stage. Secondly, two or more ova may be contained in one Graafian follicle, all of which become fertilized. Thirdly, an ovum sometimes contains more than one nucleus, a multinucleated ovum which Stöckel thinks may occur either by fusion of two or more ova or by division of the nucleus. Fourthly, an ovum may be fertilized following two successive menstrual periods, this being known as superfetation.

According to Mall,<sup>(1)</sup> the first workers (Valentine,<sup>(2)</sup> Luchart,<sup>(3)</sup> and Muller<sup>(4)</sup>), while studying the formation of twins, believed that the early primary change lies in the ovum or that twins and other polyembryos develop by division of the embryo-forming substance. Some of the later workers, who studied the

<sup>1</sup> Received for publication May 10, 1916.

developing egg, advanced the theory of polyspermy—that is, they believed that two or more sperms might fertilize an ovum, the result of which would be twins, triplets, or quadruplets, depending on the number of spermatozoa participating in the fertilization of the ovum. This, however, has never been substantiated. Moreover, since it has been shown that the chromosomes play such a definite part in cell division, the theory that twins are produced by polyspermy can be discarded.

Mall has pointed out that the first observations of any value on the development of twins and of double monsters were made by Vejkousky(5) in 1891. His experiments were made with the eggs of *Allolobophora trapezoides*, a common earthworm. He found that when the eggs were placed in water and subjected to a higher temperature segmentation began at once. At the two- or four-cell stage, however, the cells would partially separate from each other and joined twins were formed.

Driesch,(6) while working with the eggs of sea urchins, found that if the fertilized eggs were subjected to a constant temperature of 31°C. for eight hours the eggs would begin to develop. The cells would separate partially or completely from each other, each part of the divided egg developing into an embryo. If the cleavage was incomplete, joined twins were formed. Not only were twins produced, but in several instances the blastomeres separated at the four-cell stage and four embryos developed. More than four divisions were never observed. These experiments conclusively prove that two or more embryos may develop from one ovum.

Loeb,(7) likewise, produced twins experimentally with the egg of the sea urchin, but employed an entirely different method than that of Driesch. Fresh, fertilized eggs were put in an equal mixture of sea water and fresh water. Due to the difference of osmotic pressure, the eggs absorbed water so rapidly that the cell membrane ruptured and part of the protoplasm extruded. After the membrane had ruptured, the eggs were immediately placed in sea water and segmentation began. Cells wandered into the extruded protoplasm, and the development of twin embryos began. If the extruded protoplasm adhered firmly to the protoplasm within the cell, joined twins were formed, but if it became separated, two single embryos, or twins, were formed.

Wilson(8) was the first to extend this work to vertebrate eggs. While experimenting with the eggs of *Amphioxus*, he found that, by gently shaking the fertilized eggs in a solution, the blastomeres were partly or completely separated at the two-,



four-, six-, or eight-cell stage. He observed as many as eight divisions of one egg. In no case, however, was a complete embryo formed. The development was usually arrested at the gastrula stage, but sometimes went as far as the first gill-slit stage. The size of the embryos that developed from the two-, four-, six-, or eight-cell stage always retained a definite relation to the size of the normal embryo—that is, if the ovum separated in the two-cell stages, each embryo was approximately one half the size of the normal embryo. When triplets were formed, one was as large as the other two. If a cell became separated at the eight-cell stage, the embryo was about one eighth the size of a normal embryo. Wilson observed that, when the cells failed to separate completely from each other, double, triple, and quadruple monsters were formed.

The work of Newman and Patterson (9) on the development of the nine-banded armadillos undoubtedly explains the occurrence of identical twins. The unusual feature about the development of the armadillo is the fact that in one litter the embryos are always of the same sex—either all males or all females. Newman and Patterson at first believed that the embryos probably developed from one of the blastomeres of the four-cell stage, and that each embryo could, therefore, be looked upon as a lineal descendant of a blastomere. In later investigations, however, they have shown that the blastomeres do not divide at the four-cell stage, but that the young develop rather as a product of agamogenesis, belonging to the general category of budding. This budding takes place after the formation of the blastodermic vesicle. In the armadillo, then, we have normally four embryos developing from one ovum.

Werber,(11) in a recent article, has shown that, if fertilized *Fundulus* eggs are treated with a very weak solution of butyric acid or acetone, a small percentage of the ova will form joined twins while the others will develop into various kinds of monsters, some with a reduplication of parts and others that show arrested development of parts.

#### MATERIAL

In a series of approximately five hundred duck eggs that were incubated for our embryological material, four sets of twins were found. This gives a higher percentage than has been reported by other investigators who have studied chick and duck twins. One set of twins had developed to the three-somite stage; the other three sets, respectively, to the 9-, 11-, and 12-somite stage. In only one set are the embryos com-

pletely separated from each other. In the other three sets the embryos are joined along their lateral sides and would in all probabilities have developed into joined twins (Plate I, figs. 1 and 2, Plate II, and Plate III, fig. 1). In the remaining set the embryos approximate each other at the cephalic end (Plate III, fig. 2), but are separated from each other by a deep depression or groove. In the descriptions of these twins "median and lateral" will be used as if we were dealing with only one organism. "Lateral" will refer to the left side of the left component and the right side of the right component. "Median" will refer to the left side of the right embryo and the right side of the left embryo.

## SET I

These two embryos are identical in their development. The somites are rather indistinctly marked, but three or four somites can be made out. The heads of the embryos approximate each other along the lateral side, while the caudal ends are more separated, the two embryos forming an angle of about 32 degrees. Plate III, fig. 1, represents a dorsal view of the embryos. During the fixation the caudal portion of the embryos separated along the medullary grooves, forming a large, artificial gap in each embryo. The head processes are well developed and extend for a short distance over the proamnion. They are well separated from each other by a deep groove. Immediately posterior to the second dilatation of the medullary grooves the ectoderm is continuous from one embryo to the other. The medullary grooves are open in their entire extent. In the cephalic regions they have undergone differentiation and the first and second primary brain vesicles are formed. Between the two embryos there is an opacity due to the clumping of cells where the germinal layers from each embryo have met. On the ventral side of each embryo the archenteron is prolonged into the head process to form the fore-gut. The area pellucida is well defined and surrounds the embryos uniformly. Some blood-islands have developed in the area opaca.

## SET II

In set II the components are joined along the thoracic and caudal regions; this type is known as thoracopagus. The heads are separated from each other, forming an acute angle. The head processes are poorly developed, and the medullary folds are very large and have not fused at any point. Each embryo has an individual medullary groove in the region of the head; posteriorly, however, they meet to form one large, wide groove.

Each embryo is in the eight-somite stage of development. The medial somites lie immediately anterior to the medullary groove. The somites are seen much more plainly from the ventral than from the dorsal side (Plate II).

## SET III

*Dorsal side.*—These twins are in the twelve-somite stage of development and appear to be perfectly normal. The heads and caudal portion of the embryos are separated from each other, while in the thoracic region they are joined. This type of twins is known as thoracopagus. This set of twins I reconstructed, and in another paper I shall describe them in detail. The heads are parallel with each other, while the caudal ends are separated, forming an angle of about 45 degrees. Ventral to the head processes is the proamnion, which is thin and transparent. The area pellucida regularly surrounds the embryos on all sides. The cephalic portions of the embryos are uniformly developed. On the tip of each head is a depression—the anterior neuropore. The medullary grooves are closed except for two small portions—one in the region of the somites, and the other along the caudal portion of each embryo. The latter portion is quite wide and expanded at the distal end. The fore- and hind-brain are separated from each other by a slight constriction, the former being considerably more expanded in a lateral direction. The hind-brain has about the same length as the combined length of the fore- and mid-brain. Between the two embryos in the cardiac region is a saccular protuberance into which blood vessels empty. The omphalomesaraic veins and a large median vein are the principal veins that empty into the primitive heart.

*Ventral side.*—The head processes can be distinctly seen through the proamnion. Posterior to the heads the entoderm is thrown into a fold which forms a right angle with the axis of the embryo. These folds are caused by the omphalomesaraic veins as they approach the embryos and terminate in the cardiac region. Immediately posterior to the omphalomesaraic veins is the posterior opening of the fore-gut or the fovea cardiaca. The two openings are separated from each other by a ventral bulging which is formed by the median vein as it approaches the embryo from a caudal direction (Plate I, figs. 1 and 2).

## SET IV

The bodies of these two embryos lie in the same linear axis. One component is in a fairly good state of preservation, while



the cephalic portion of the other is somewhat broken down. These embryos are in the twelve-somite stage of development. The medullary tubes are completely closed in the region of the head and thorax, while in the caudal region they are still open posterior to the sixth somite.

The embryo on one side shows a marked disintegration along the cephalic end. The caudal end is in a better state of preservation; here the medullary groove is still open. No somites appear along the lateral sides of the medullary groove. The embryos are separated from each other by a cleft, which is clearly seen on the cephalic end of one embryo (Plate III, fig. 2).

#### DISCUSSION

The underlying causes involved in the formation of twins and other polyembryos are still somewhat obscure; however, with the experimental work that has been done in the production of polyembryony and the large amount of literature that has accumulated on the subject, we are now able to arrive at some fairly definite conclusions. It has been definitely shown that in some of the anamniotes twins, triplets, quadruplets, etc., can be produced by mechanical and chemical changes. These are definite results that have been obtained by altering the environment by external agents. If the intrinsic factors be considered, little imagination is needed to conceive of distorted embryos, twins, etc., as being due to faulty metabolism or to some physical law that is so wrapped up in physicochemical processes that it is still impossible to solve the mystery.

Investigators have attempted to produce chick and duck twins and certain types of monsters by shaking the eggs, raising and lowering the temperature, or varnishing the eggshells. Results, however, have been uncertain, and at no time could the experimenter foretell what kind of twins or monstrosity would develop after treating the egg by one of the above methods.

For convenience, twins should be divided into three main classes—(a) dissimilar twins, (b) identical twins, and (c) joined twins. The first class includes twins of different sex or unlike in their general development and physiognomy. This type of twins results from the fertilization of several ova or possibly a multinucleated ovum. The second class includes those embryos that are identical in almost every detail—the result, undoubtedly, of an early cleavage of the blastomeres or a complete separation of the fertilized egg at some later stage, as Newman and Patterson have shown in the nine-banded arma-



dillos. The third class includes all those twins that are joined—probably due to an incomplete separation of the early blastomeres or of the embryo-forming substance at some later stage. To this type belong the Siamese twins, who were joined to each other along the ventral part of their abdomen. Wilder<sup>(12)</sup> has shown that identical and joined twins belong to the same category. In a series of schematic drawings he has shown that in the Janus type the twins may be attached to each other from their head to the lower part of the abdomen, or they may be separated from above downward until the attachment grows less and less and finally each embryo has a separate umbilical cord, thus resulting in the formation of true identical twins.

It seems evident, therefore, that the factor which is present in the production of identical twins must likewise be present in the formation of joined twins. We can conceive of a determiner that regulates the moulding of the body into a certain form and type; further, that this determiner divides equally in the first stages of segmentation—in the nine-banded armadillos as far as the blastula stage when the division of the fertilized ovum first begins and a set of identical quadruplets develops.

In the four sets of duck twins, three sets were joined while in the remaining one the twins were separated. To say that the last set belongs to the first class or to the second class would be mere speculation, as it may answer the description of either type. Not until further experiments have been carried out on the development of twins in amniotes can we say positively that a set of twins was produced by an extrinsic or an intrinsic modification of the environment. The other three sets of joined twins undoubtedly developed from a single, fertilized ovum, with only a partial separation of the early blastomeres, or at a slightly later stage in the process of development.

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## ILLUSTRATIONS

[Drawings by J. Castro.]

### PLATE I

- FIG. 1. Dorsal view of duck twins, set III. *pa*, proamnion; *ap*, area pellucida; *mg*, *mg'*, medullary groove; *np*, anterior neuropore; *ao*, area opaca; *cr*, cardiac region; *cmg*, caudal portion of medullary groove still open.
2. Ventral view of duck twins, set III. *ao*, area opaca; *fc*, fovea cardiaca; *ompv*, *ompv'*, omphalomesaraic vein; *mv*, median vein between the two embryos; *som*, somites.

### PLATE II

Dorsal view of duck twins, set II. *ao*, area opaca; *pa*, proamnion; *hp*, *hp'*, head process; *ap*, area pellucida; *som*, *som'*, somites; *mg*, medullary groove; *cmg*, medullary groove in common.

### PLATE III

- FIG. 1. Dorsal view of duck twins, set I. *ao*, area opaca; *ap*, area pellucida; *hp*, *hp'*, head process; *nc*, *nc'*, notochord; *fl*, line of fusion between the two embryos.
2. Dorsal view of duck twins, set IV. *ap*, area pellucida; *ao*, area opaca; *hp*, head process; *lf*, line of fusion between two embryos; *hpd*, head process disintegrated; *mg*, medullary groove; *som*, somites.





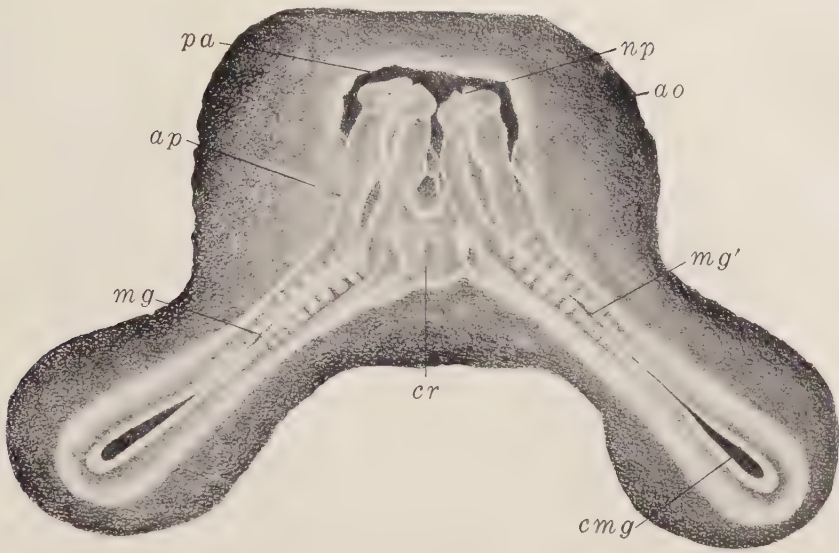


Fig. 1. Dorsal view of duck twins, set III.

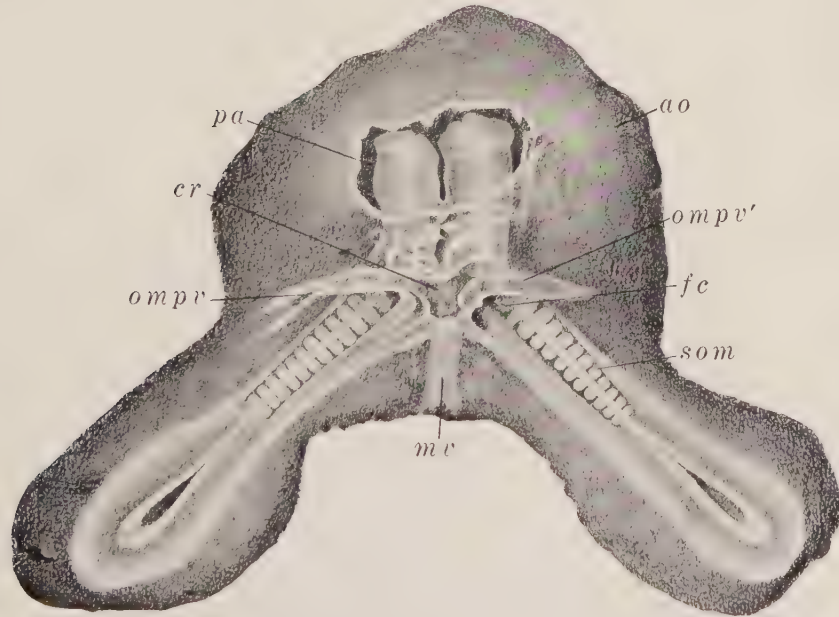
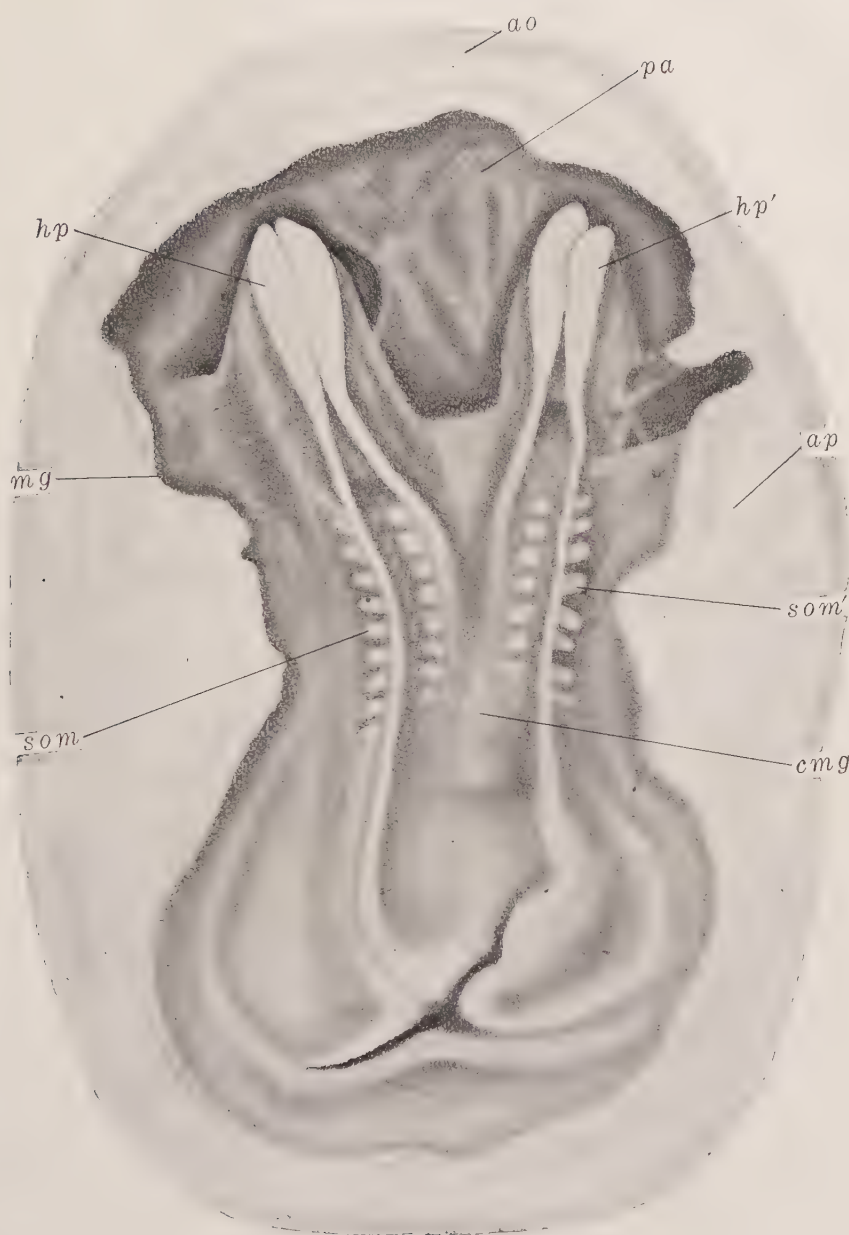


Fig. 2. Ventral view of duck twins, set III.





DORSAL VIEW OF DUCK TWINS, SET II.

PLATE II.





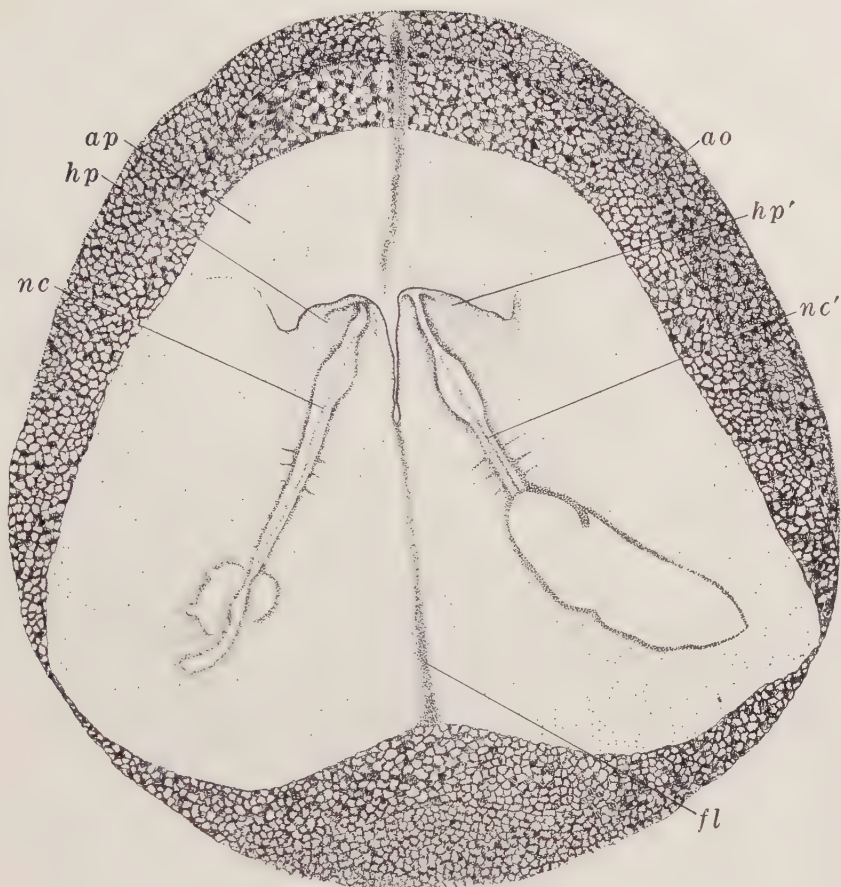


Fig. 1. Dorsal view of duck twins, set I.

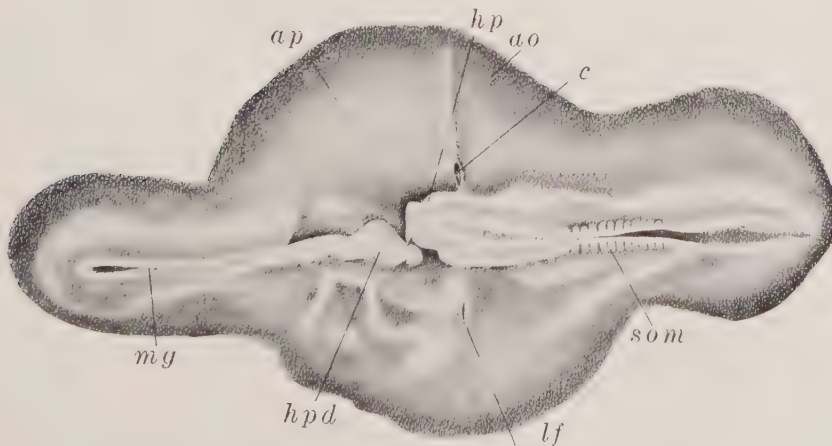


Fig. 2. Dorsal view of duck twins, set IV.



# THE INFLUENCE OF FRESH AND AUTOCLAVED COWS' MILK ON THE DEVELOPMENT OF NEURITIS IN ANIMALS <sup>1</sup>

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## TWO PLATES AND 2 TEXT FIGURES

The rapidly increasing use for infant feeding of canned whole cows' milk in the Philippine Islands has suggested that we ought to obtain some information as to whether or not the antineuritic vitamins of milk are destroyed by heating. This is particularly true, because of the large number of cases of infantile beriberi that occur here and the probable existence of many cases of latent infantile beriberi which may easily become acute.<sup>2</sup>

We had expected that long-continued experimental observations on dogs and pigs fed on autoclaved milk would be complicated by the development of scorbutic symptoms, for it is generally believed that the antiscorbutic principles are easily rendered ineffective by heat, and numerous observations have been recorded indicating that heated milk induces scurvy in infants.<sup>3</sup> Hess and Fish<sup>4</sup> have reported that they have even seen several cases of infantile scurvy as the result of feeding milk which had been Pasteurized at 63°C. for only thirty minutes. Scurvy may be produced experimentally in animals. Bolle<sup>5</sup> found that guinea pigs fed on boiled milk developed scurvy, while those given raw milk did not. His experimental findings were not confirmed by Bartenstein.<sup>6</sup> Hart<sup>7</sup> fed monkeys on canned milk, and they become typically scorbutic. Schmorl<sup>8</sup> obtained scorbutic changes in dogs given a diet deficient in phosphates. Holst and Fröhlich<sup>9</sup> have observed typical scurvy in guinea pigs by feeding oats, and Ingier<sup>10</sup> has produced scorbutic

<sup>1</sup> Received for publication May 31, 1916.

<sup>2</sup> Saleeby, Proceedings of the Philippine Islands Medical Association, *This Journal*, Sec. B (1915), 10, 87.

<sup>3</sup> Cf. Funk, *Ergeb. d. Physiol.* (1913), 13, 125.

<sup>4</sup> *Am. Journ. Dis. Child.* (1914), 8, 385.

<sup>5</sup> *Zeitschr. f. diät. u. physik. Therapie* (1903), 6, 354.

<sup>6</sup> *Jahrb. f. Kinderh.* (1905), 61, 6.

<sup>7</sup> *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.* (Virchow) (1912), 208, 367.

<sup>8</sup> *Arch. f. exp. Path. u. Pharm.* (1913), 73, 313.

<sup>9</sup> *Zeitschr. f. Hyg. u. Infektionskrankh.* (1912), 72, 1.

<sup>10</sup> *Journ. Exp. Med.* (1915), 21, 525.

conditions in foetal and newborn guinea pigs. Fröhlich<sup>11</sup> states that milk heated to 98°C. for ten minutes will not prevent scurvy in guinea pigs fed on oats though fresh milk is effective; fresh milk given alone may produce scorbutic symptoms.

Rickets, infantile scurvy, scurvy, and even pellagra have been ascribed, along with beriberi, to the consumption of vitamine-deficient and so-called "devitalized" foods, the protective substances of which have been destroyed by long storage or heating (cooking, canning, etc.).<sup>12</sup> Darling<sup>13</sup> asserts the intimate relationship between scurvy and beriberi. He observed well-marked right-sided hypertrophy of the heart and degenerative changes in the cardiac muscle and the pneumogastric nerve. Typical beriberi conditions, such as cardiac hypertrophy and oedema, have been described recently by Hess<sup>14</sup> as part of the symtomatology and pathology of infantile scurvy. Holst and Fröhlich and also Ingier demonstrated degenerative changes in the nerves of some of their animals. Holst<sup>15</sup> observed that pigs may develop symptoms intermediate between beriberi and scurvy.

Very little satisfactory experimental evidence has been obtained as to the effect of heat on the antineuritic substances proper; such experiments as have been reported indicate that the vitamine is affected. Thus Fraser and Stanton<sup>16</sup> have shown that the antineuritic properties of rice polishings are destroyed if autoclaved for two hours. Grijns<sup>17</sup> states that dogs fed on autoclaved meat develop neuritis. Grijns also found that unpolished rice may lose its protective power against beriberi when heated to a temperature of 130°C. In the isolation of the vitamine substances or in the preparation of tiqui-tiqui extracts, it is the practice to avoid heating except in acidified solution; otherwise the protective substances may be destroyed.<sup>18</sup>

The antineuritic properties of milk have not been thoroughly investigated. Vedder and Clark<sup>19</sup> have reported experiments in which 5 cubic centimeters of fresh cows' milk were given daily to four fowls fed on polished rice; two of these died of

<sup>11</sup> *Zeitschr. f. Hyg. u. Infektionskrankh.* (1912), 72, 155.

<sup>12</sup> Funk, *Die Vitamine*, Wiesbaden (1914); also cf. Schaumann, *Arch. f. Schiffs- u. Trop.-Hyg.* (1915), 19, 393, 426.

<sup>13</sup> *Journ. Am. Med. Assoc.* (1914), 63, 1290.

<sup>14</sup> *Ibid.* (1915), 65, 1003.

<sup>15</sup> Cf. Schaumann, *Arch. f. Schiffs- u. Trop.-Hyg.* (1914), 18, Beiheft 6.

<sup>16</sup> Studies from the Institute for Medical Research. Federated Malay States (1911), No. 12.

<sup>17</sup> Cited from Schaumann, loc. cit.

<sup>18</sup> Vedder and Williams, *This Journal, Sec. B* (1913), 8, 175.

<sup>19</sup> *Ibid.* (1912), 7, 423.



neuritis in a short time, while the remaining two were healthy after two months. One of us<sup>20</sup> has fed fowls daily 20 cubic centimeters of normal human milk along with polished rice; the birds developed typical neuritis. Cooper<sup>21</sup> found that 35 cubic centimeters of cows' milk given with polished rice to pigeons merely delayed the appearance of the neuritis. Funk and Macallum<sup>22</sup> claim to have shown chemically the presence of vitamine in milk.

It would seem that antineuritic properties, if present in milk, exist only in very small amounts. In none of the experiments mentioned above has milk prevented the development of neuritis. Accordingly our work has been directed to ascertain by feeding experiments whether or not antineuritic substances were present in milk and to what extent these would be affected by autoclaving the milk for two hours at 125°C. The first observations were made on fowls; subsequently experiments were carried on with young dogs and pigs.

#### EXPERIMENTS WITH FOWLS

Three well-grown young male fowls were fed 40 grams of polished rice and were given by hand 100 cubic centimeters of fresh cows' milk daily. A second set of three fowls received 40 grams of polished rice and 100 cubic centimeters of the same milk, which had been autoclaved at 125°C. for two hours. Rice, not consumed by the fowls, was fed by hand. The experiment is summarized in Table I.

TABLE I.—*Fowls fed on fresh and autoclaved milk.*

[The fowls received 100 cubic centimeters of milk and 40 grams of rice daily.]

Fowl No.	Milk.	Neuritis.	Gain.	Remarks.
		Days.	Grams.	
1	Fresh.....	31	319	A slight spasticity was observed intermittently from the thirty-first day.
2	do .....	31	235	
3	do .....	86	353	
4	Autoclaved.....	62	500	Died on the thirty-sixth day.
5	do .....		166	
6	do .....	31	168	

The experiment shows that the administration of 100 cubic centimeters of fresh cows' milk daily did not prevent the develop-

<sup>20</sup> Gibson, *ibid.* (1913), 8, 469.

<sup>21</sup> *Journ. Hyg.* (1913), 7, 268; *ibid.* (1914), 14, 12.

<sup>22</sup> *Biochem. Journ.* (1914), 7, 356; *Journ. Chem. Soc.* (1914), 104, II, 805.

ment of polyneuritis in the three fowls of this series—in two of them in thirty-one days, about the same time in which the neuritis would be expected to appear if the polished rice had been given alone. Of the autoclaved-milk-fed fowls, one developed neuritis on the sixty-second day, one died suddenly on the thirty-sixth day from causes not revealed at autopsy, and the third came down with neuritis on the thirty-first day. The results indicate that the milk has little or no protective effect against polyneuritis, and that the autoclaving of the milk has not promoted the onset of the neuritic symptoms.

The fowls showing symptoms of neuritis were killed shortly before the time of probable death, and they were then autopsied. In each case a segment of the sciatic nerve was removed and was fixed and stained with osmic acid (Marchi). The nerves of all six fowls showed typical Wallerian degeneration.

Funk and Douglass<sup>23</sup> have shown that there is marked atrophy of the glands of internal secretion in pigeons suffering from rice polyneuritis; the thymus gland, in particular, is affected. Four of the above fowls were examined by Dr. B. C. Crowell, of the College of Medicine and Surgery, for persistence of the thymus gland. The thymus gland of one fowl (No. 6) was small, while the glands of the other three fowls (Nos. 1, 3, and 4) were large. Partly from the findings in the case of these fowls, Crowell<sup>24</sup> concludes correctly that there is apparently no fundamental connection between beriberi and the atrophy of the thymus. However, we are of the opinion that the addition of milk to the diet of polished rice has supplied some nutritive deficiency, which does have an intimate relationship to the endocrin glands though not influencing the development of the neuritis.

The fowls fed on autoclaved milk continued to increase in weight as did those given the fresh milk. While the development of polyneuritis in fowls is usually accompanied by a marked loss of body weight, this symptom is not characteristic, and fowls may even gain in weight.<sup>25</sup>

The neuritis observed in these fowls has been of the mild type; with not one of the five fowls have we obtained the fulminating attacks of polyneuritis so frequently observed when polished rice alone is given.<sup>26</sup>

<sup>23</sup> *Journ. Physiol.* (1914), 47, 475.

<sup>24</sup> Williams and Crowell, *This Journal*, Sec. B (1915), 10, 121.

<sup>25</sup> Gibson, *ibid.* (1913), 8, 351; and Schaumann, *loc. cit.*

<sup>26</sup> Vedder and Clark, *ibid.* (1912), 7, 423.

In the following experiment fowls were given 200 cubic centimeters of milk and 10 (later 20) grams of rice daily.

TABLE II.—*Fowls fed on fresh and autoclaved milk.*

[The fowls received 200 cubic centimeters of milk and 10 (later 20) grams of rice daily.]

Fowl No.	Milk.	Neuritis.	Gain.	Remarks.
			<i>Grams.</i>	
1	Fresh.....	[None.....	360	Killed, seventy-ninth day.
2	.....do.....	.....do.....	548	Do.
3	.....do.....	.....do.....	277	Killed, fiftieth day.
4	.....do.....	.....do.....	46	Killed, twentieth day.
5	.....do.....	.....do.....	16	Died, fifty-sixth day.
6	.....do.....	.....do.....	390	Killed, one hundred ninety-second day.
7	Autoclaved.....	.....do.....	167	Do.
8	.....do.....	.....do.....	685	Do.

Two hundred cubic centimeters of milk per diem, either fresh or autoclaved, have protected fowls against polyneuritis when given with from 10 to 20 grams of rice daily. It must be said that the rice used in this experimental series did not have all of the pericarp removed in polishing. We have demonstrated in a previous paper <sup>27</sup> that the peripheral nerves in fowls will show degenerative changes histologically, without symptoms of polyneuritis, even when they are fed on palay (unhusked rice). When the nerves of the eight milk-fed fowls were examined, no evidence of degeneration was found.

These observations on fowls may be summed up as follows:

Fowls fed on 100 cubic centimeters of either fresh or autoclaved milk and 40 grams of rice daily developed typical polyneuritis of the mild type in from thirty-one to eighty-four days. All the fowls regularly increased in body weight throughout the experiment. The fowls showed little or no atrophy of the thymus gland, as reported by Funk for pigeons fed on polished rice alone.

Fowls fed on 200 cubic centimeters of either fresh or autoclaved milk and not over 20 grams of rice daily did not develop neuritis; moreover there was no evidence of degenerative changes in the peripheral nerves of these fowls.

Therefore milk seems to contain only a small amount of vitamine, which is not destroyed by autoclaving for two hours at 125°C.

<sup>27</sup> Ibid. (1914), 9, 119.

## EXPERIMENTS WITH DOGS

Experimental deficiency neuritis has been produced in dogs.<sup>23</sup> Accordingly we have made observations on five dogs of the same litter, three of which received milk autoclaved for two hours at 125°C and two of which received the fresh milk. As in the experiments with fowls, daily morning deliveries of fresh cows' milk were obtained from a local dairy. The dogs were kept in a large pen outdoors. They were fed individually, and a record was kept of the amount eaten by each animal. The dogs were 17 days old when the experiment was started and weighed between 593 and 725 grams. The results of the experiment are given in Table III.

TABLE III.—*Dogs fed on fresh and autoclaved milk.*

Dog.		Milk.	Neuritis. <sup>a</sup>	Gain on 47th day.	Remarks.
No.	Sex.				
1	Female	Fresh	Positive	Grams. 1,197	Paralysis of posterior limbs, fifty-fifth to fifty-ninth day; pronounced oedema of limbs, eighty-ninth day until killed on the ninety-second day. Total gain in weight, 2,860 grams.
2	do	do	do	1,592	Killed, forty-eighth day.
3	Male	Autoclaved	do	1,370	Do.
4	do	do	do	1,222	Marked cedema of limbs, ninetieth day; killed, ninety-second day. Total gain in weight, 2,145 grams.
5	Female	do	do	1,345	Killed, forty-eighth day.

<sup>a</sup> As evidenced by histological examination (Marchi) of the sciatic nerve.

On the forty-seventh day of the experiment all the dogs were in excellent condition. They were slightly undersized as compared with another dog of the same litter which had been allowed to suckle the bitch, but at this time was also being given mixed food. Three dogs (Nos. 2, 3, and 4) were chloroformed on the forty-eighth day. The experiment was continued with dogs 1 and 6. Toward the conclusion of the experiment it became difficult to persuade the two dogs to eat, although fair amounts of milk were taken until the onset of oedema on the eighty-ninth day of the experiment. At this time the dogs were somewhat thin, undersized, and partially aphonic and refused

<sup>23</sup> Cf. Schaumann, loc. cit.



to eat the milk. In as much as starvation contributes to the degenerative change in the peripheral nerves,<sup>29</sup> the dogs were chloroformed on the ninety-second day.

No scorbutic symptoms were observed in any of the dogs in the course of the experiment. No gross changes were found at autopsy. The endocrin glands appeared normal. Histological examination of the sciatic nerves revealed degenerative changes for the two dogs fed on fresh cows' milk as well as for the three given autoclaved milk.

Differences in the rates of growth and the food consumed were

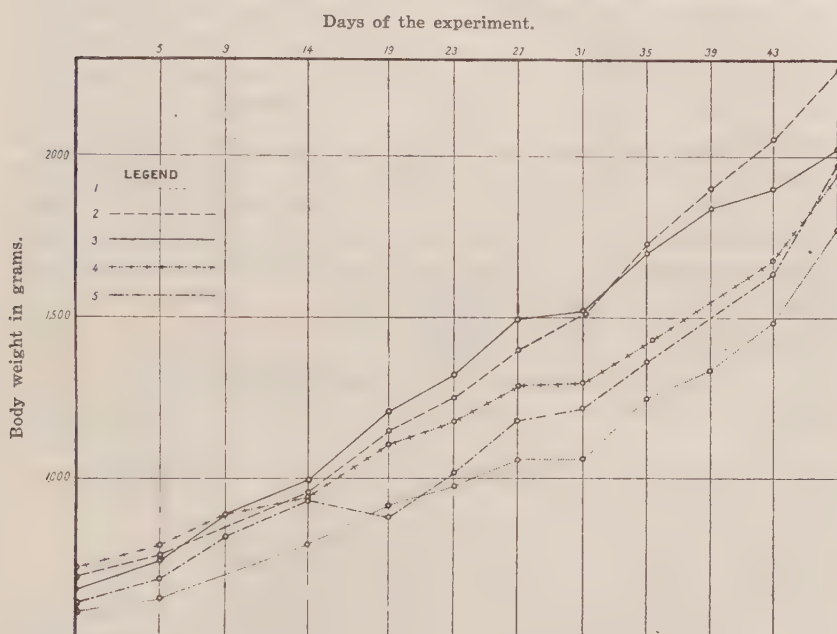


FIG. 1. Growth curves for dogs fed on fresh and autoclaved milk.

not great between the dogs fed on fresh and those fed on autoclaved milk; they were slightly in favor of the former. The growth changes will be discussed later in this paper.

The dogs soon lost their appetite for the cows' milk, particularly for the autoclaved milk. The addition of bone ash (5 grams daily) to the milk of dogs 1 and 6 after the fiftieth day led to better consumption and utilization of the milk. The dogs of each group seemed ravenous when tempted with beef bones, the more so the longer the experiment was continued.

Our experiment with dogs has shown that exclusive feeding

<sup>29</sup> Chamberlain, Bloombergh, and Kilbourne, *This Journal, Sec. B* (1911), 6, 177.

with either fresh or autoclaved milk induced degenerative changes in the peripheral nerves of three dogs at the end of forty-seven days. Degeneration of the peripheral nerves, œdema, and partial aphonia were observed subsequently in the one fresh-milk dog and the one autoclaved-milk dog remaining. Little difference could be observed between the effect of the diet on the two dogs given fresh milk and the three receiving autoclaved milk. No scorbutic symptoms were observed.

#### EXPERIMENTS WITH PIGS

Six 17-day old pigs of the native breed were selected from the same litter and three were fed exclusively on fresh cows' milk and three on autoclaved milk. The pigs were kept in separate pens. One fresh-milk pig and one autoclaved-milk pig were killed after thirty-four days; a similar pair were killed after sixty days. Of the remaining pigs, the autoclaved-milk pig died on the seventy-fourth day of the experiment, and the fresh-milk pig on the one hundred sixth day. As in the earlier observations on dogs, the milk eaten was recorded daily and the body weights were recorded every four days.

The results are given in Table IV.

TABLE IV.—*Pigs fed on fresh and autoclaved milk.*

Fig.		Milk.	Neuritis, <sup>a</sup>	Weight.		Remarks.
No.	Sex.			Initial.	Gain.	
				<i>Grams.</i>	<i>Grams.</i>	
1	Female	Fresh	Positive	820	3,730	Killed, thirty-fourth day.
2	do	Autoclaved	do	800	3,700	Do.
3	Male	Fresh	do	776	6,274	œdema of limbs and face from the fifty-sixth day until killed on the sixtieth day.
4	do	Autoclaved	do	824	7,526	Do.
5	do	Fresh	do	550	<sup>b</sup> 15,890	œdema of limbs and face from about the fifty-sixth day and gradually increasing aphonia. Died on the one hundred sixth day from ruptured coronary artery.
6	Male	Autoclaved	do	550	10,265	œdema of limbs and face from about the fifty-sixth day and gradually increasing aphonia. Died on the seventy-fourth day from ruptured coronary artery.

<sup>a</sup> As evidenced by histological examination (Marchi) of the sciatic and pneumogastric nerves.

<sup>b</sup> This pig had increased in weight by 10,150 grams on the day on which the last figure for the companion pig (No. 6) was obtained.

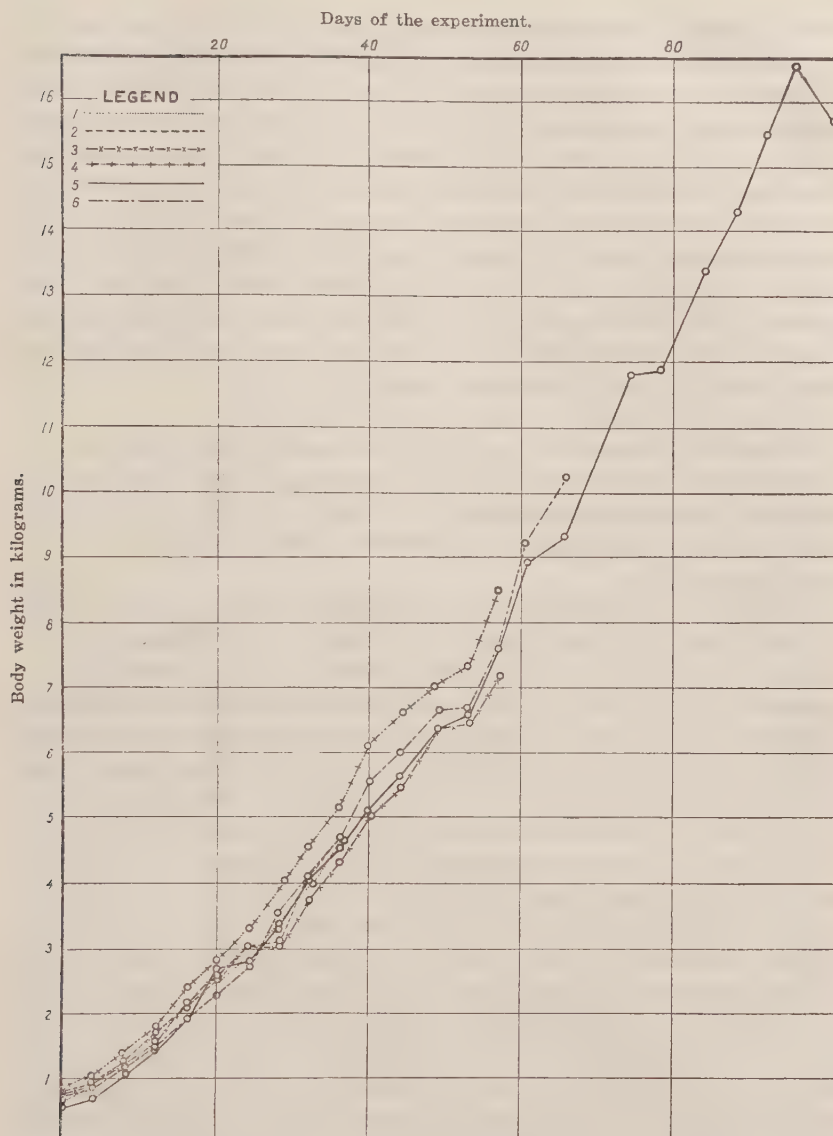


FIG. 2. Growth curves for pigs fed on fresh and autoclaved milk.

The results are remarkably concordant for the fresh-milk and the autoclaved-milk pigs. All the pigs were apparently normal on the thirty-fourth day when pigs 1 and 2 were killed.<sup>30</sup>

<sup>30</sup> It is to be regretted that the experiment could not have been done on a larger scale so that a greater number of pigs could have been observed over both short and long periods. It is very difficult to obtain large quantities of fresh milk in the Philippines, and it was questionable at times as to whether we could get enough for the experiment as actually conducted.

All the pigs had increased in weight at this time to an amount only a little less than that of a single pig, which had been left with the sow. Autopsy of pigs 1 and 2 showed that the animals were seemingly normal. The endocrin glands were well developed. Careful examination of the gums, periostial membranes, and bones gave no evidence of the existence of any scorbutic condition. Histological examination of the sciatic and pneumogastric nerves showed that degenerative changes had taken place; the sciatic was affected to the greater degree. The sciatic of the fresh-milk-fed pig showed more extensive degeneration than that of the pig given autoclaved milk, as may be seen from the accompanying reproduction of the microphotographs of the Marchi preparations (Plate II).

Almost simultaneously on the fifty-sixth day of the experiment it was observed that all of the remaining pigs had developed a noticeable œdema of the limbs and face. The pigs, otherwise, were in excellent condition; they ate well and were growing rapidly.

Little or no difference could be detected between those receiving the fresh milk and those on the autoclaved milk on the sixtieth day of the experiment. The œdema was still evident. The organs of pigs 3 and 4, which were chloroformed and autopsied, were practically normal. Careful examination gave no evidence of scurvy. The tissues of the limbs were moist from the œdema. The endocrin glands seemed well developed and normal. As in the case of the previous pair of pigs, the sciatic nerves had undergone pronounced degenerative changes; the pneumogastrics were much less affected.

The experiment was continued with the two remaining pigs. These continued to increase in weight rapidly. The œdema of the extremities was intermittently noticeable, and there was a gradually increasing condition of aphonia. On the morning of the seventy-fourth day pig 6 died suddenly. On autopsy much fluid was found in the peritoneal and pleural cavities and the pericardial sack was dilated with clotted blood. Examination of the heart showed that a rupture of the anterior coronary artery had occurred. Otherwise the pig was apparently normal. Examination of the sciatic nerve showed a pronounced degeneration; the pneumogastric was much less affected. When weighed three days earlier, pigs 5 and 6 weighed 10,700 and 11,000 grams, respectively. Pig 5 was found dead on the morning of the one hundred sixth day of the experiment; the body was still warm. Autopsy gave almost the identical picture described for pig 6, the rupture of the coronary vessel occurring in



the apical region and being apparently of longer standing. The heart was elongated with a hypertrophied left ventricle. As with pig 6, there was no evidence of scorbutis. Examination of the sciatic and pneumogastric nerves showed degenerative changes as described for pig 6.

The experiment with pigs has corroborated the results which we have obtained with fowls and dogs. No effects due to heating the milk have been observed. The results for each pig of each pair have been strikingly similar—that is, continued and practically normal growth; no evidence of scorbutic symptoms; degeneration of the sciatic nerve in every instance and to a less degree of the pneumogastric nerve; œdema in the pair killed at the end of sixty days; and for the remaining pair, œdema, aphonia, and death from rupture of the anterior coronary artery, which had broken through the cardiac musculature. However, we hesitate to attribute the cause of death in these two pigs to the milk diet without further experimental confirmation.

#### GROWTH OF DOGS AND PIGS FED ON FRESH AND ON AUTOCLAVED COWS' MILK

The literature on the effects of boiled milk on the development of young animals has been reviewed by Lane-Clapton.<sup>31</sup> She concludes that animals and infants develop better on milk of their own species, whether this milk is boiled or raw; that on milk of foreign species development is best on boiled milk; and that it is by no means proved that infants develop scurvy from boiled milk alone. We shall discuss our results briefly in the light of later investigations of conditions which influence growth.

Recent experimental work has indicated that growth processes are stimulated by accessory nutritive principles contained in the food. Young animals fed on artificial mixtures of pure salts, carbohydrates, fats, and complete proteins do not grow. Hopkins<sup>32</sup> has shown that the addition of small amounts of milk to such a diet leads to immediate and normal growth. Funk<sup>33</sup> has reported that young chickens fed on unpolished rice similarly do not increase in size; unpublished experiments on young chickens and rabbits have led us to the same conclusion. Osborne and Mendel<sup>34</sup> have shown that the growth principle in cows' milk for rats is associated with the milk fats and occurs

<sup>31</sup> *Ergebn. d. inn. Med. u. Kinderh.* (1913), 635; Report to the Local Government Board, London (1912), n. s. No. 63, 1219.

<sup>32</sup> *Journ. Physiol.* (1912), 44, 425.

<sup>33</sup> *Zeitschr. f. physiol. Chem.* (1914), 88, 352.

<sup>34</sup> *Journ. Biol. Chem.* (1915), 20, 379.

principally in the liquid oil from which the fats with higher melting point have been separated. This rôle of fats in promoting growth has been confirmed by McCollum and Davis.<sup>35</sup> Funk and Macallum<sup>36</sup> consider that their experiments show the "inability of either butter or butter fat to stimulate the growth of young rats;" yeast contains a growth-promoting principle, presumably a vitamine. McCollum and Davis<sup>37</sup> conclude that there is an accessory water-soluble, growth-promoting substance in milk whey, wheat embryo, and egg yolk.

The growth-promoting substances are apparently not influenced by heating. Osborne and Mendel<sup>38</sup> find that heating butter fat for two and one-half hours was without destructive effect. McCollum and Davis<sup>39</sup> state that milk whey can be kept at a boiling temperature for six hours without appreciable loss of activity of the accessory water-soluble, growth-promoting substance in milk whey; they also furnish evidence that this substance (present in lactose preparations) is not destroyed by autoclaving. They ascribe the loss of the growth-accelerating property of heated milk to changes in the casein which render it nutritively inadequate.<sup>40</sup>

Our own experiments with dogs and particularly with pigs have indicated that autoclaving the milk for two hours at 125°C. has not affected appreciably either the growth-promoting principles of the milk or the nutritive value of the caseinogen. Records for forty-seven days of the experiment with dogs showed that it required 12.4 and 12.1 cubic centimeters of milk for each increase of 1 gram of body weight for the two puppies fed on fresh milk; for the three given autoclaved milk the figures are 13.1, 13.5, and 12.8 cubic centimeters. For an increase of 1 gram in body weight of the pigs killed on the thirty-fourth day, there were required 9.1 and 9.2 cubic centimeters of fresh and autoclaved milk, respectively; similarly for the pair of pigs killed the sixtieth day, 10.7 and 10.5 cubic centimeters, respectively; and for the remaining pair of pigs, 11.2 and 10.0 cubic

<sup>35</sup> Ibid. (1914), 19, 214.

<sup>36</sup> Ibid. (1915), 23, 413; see also MacArthur and Luckert, *ibid.*, 20, 161, and Bosworth, Bowditch, and Ragle, *Am. Journ. Dis. Child.* (1915), 9, 81.

<sup>37</sup> *Journ. Biol. Chem.* (1916), 23, 181, 247.

<sup>38</sup> Loc. cit.

<sup>39</sup> Loc. cit.

<sup>40</sup> Daniels and Stuessy, *Am. Journ. Dis. Child.* (1916), 11, 45, have reported very recently that rats fed on boiled milk grow to about half their normal size. Lane-Clapton, *Journ. Hyg.* (1909), 9, 233, has rather conclusively shown that there is no diminution of the nutritive value of milk heated to boiling or dried (120°C.) when fed to young rats.

centimeters, respectively. The growth curves for the dogs and pigs are given in the accompanying charts.

Our experiments have indicated that the antineuritic vitamine is present in milk in slight amounts only and that the continued feeding of either fresh milk or autoclaved milk to animals (dogs and pigs) without suitable additions to the diet induces certain beriberi symptoms—namely, degeneration of the peripheral nerves, persistent cedema, and aphonia. There is no evidence that autoclaving the milk for two hours has in any way affected its nutritive value. Not even scorbutic changes have been observed. At the best, the antineuritic powers of the milk are so slight that in infant feeding the diet should be extended as soon as possible. The young of healthy mothers probably come into the world with a reserve supply of the vitamine substances sufficient to tide them over nutritively until the time when, under natural conditions of life, they would begin to eat other foodstuffs.





## ILLUSTRATIONS

### PLATE I

- FIG. 1. Pig 1, fed on fresh milk, killed on the thirty-fourth day.  
2. Pig 2, fed on autoclaved milk, killed on the thirty-fourth day.  
3. Pig 3, fed on fresh milk, killed on the sixtieth day.  
4. Pig 4, fed on autoclaved milk, killed on the sixtieth day.

### PLATE II

- FIG. 1. Pig 1, fed on fresh milk, photomicrograph of the sciatic nerve (Marchi).  
2. Pig 2, fed on autoclaved milk, photomicrograph of the sciatic nerve (Marchi).

### TEXT FIGURES

- FIG. 1. Chart, showing growth curves for dogs fed on fresh and autoclaved milk. Curves 1 and 2 are for the fresh-milk-fed dogs, and curves 3, 4, and 5 are for the autoclaved-milk-fed dogs. Days are given as abscissæ and body weights in grams as ordinates. The chart is for the forty-seven days of the experiment only.  
2. Chart, showing growth curves for pigs fed on fresh and autoclaved milk. Curves 1, 3, and 5 are for the fresh-milk-fed pigs and curves 2, 4, and 6 are for the autoclaved-milk-fed pigs. Days of the experiment are given as abscissæ and body weights in kilograms as ordinates.





Fig. 1. Pig 1, fresh milk, killed the 34th day.



Fig. 2. Pig 2, autoclaved milk, killed the 34th day.



Fig. 3. Pig 3, fresh milk, killed the 60th day.



Fig. 4. Pig 4, autoclaved milk, killed the 60th day.

PLATE I.





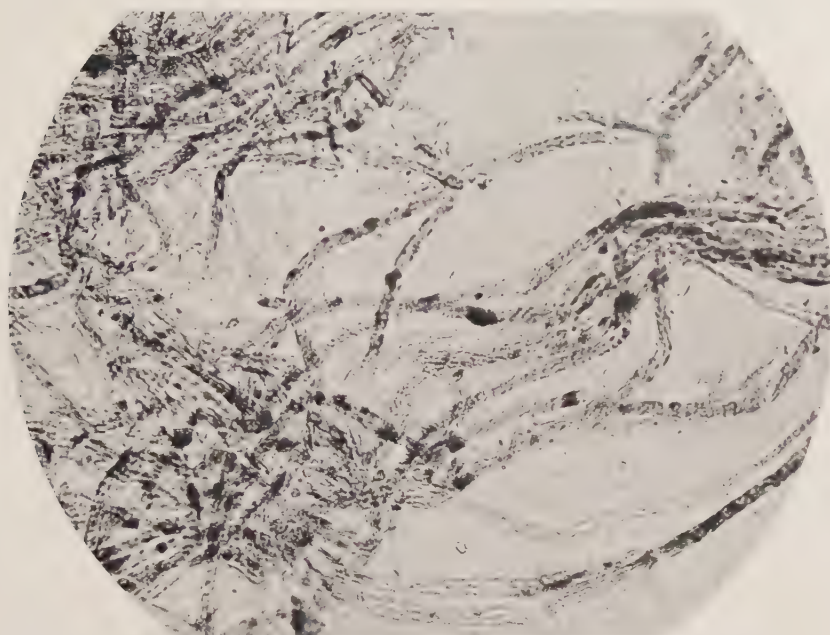


Fig. 1. Pig 1, fresh milk, photomicrograph of the sciatic nerve (Marchi).

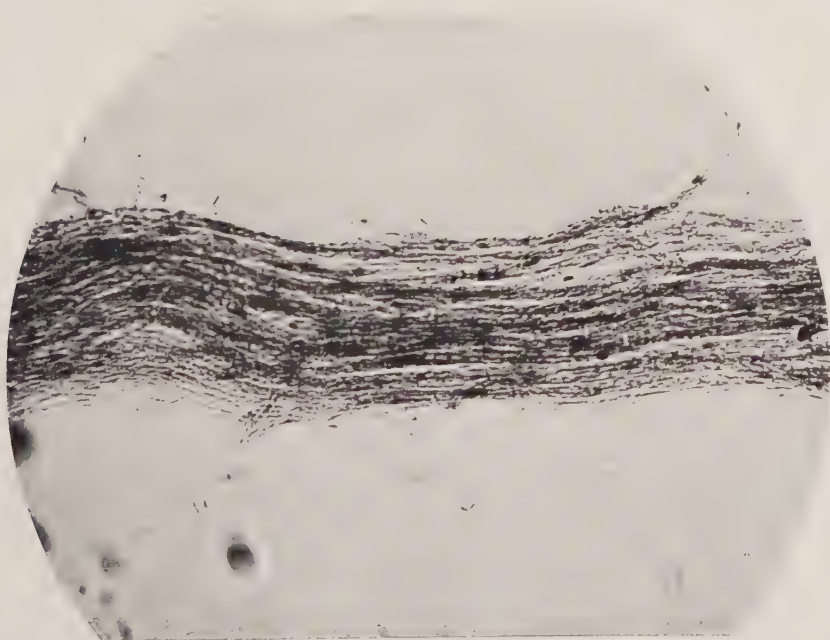


Fig. 2. Pig 2, autoclaved milk, photomicrograph of the sciatic nerve (Marchi).

PLATE II.



## BLOOD-PRESSURE PICTURE OF THE FILIPINOS<sup>1</sup>

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THREE PLATES AND 2 TEXT FIGURES

### INTRODUCTION

It is only in very recent years that the sphygmomanometer (Plate I) has come into general use in practical medicine as well as in the laboratory. It is already generally believed to furnish a great amount of diagnostic, prognostic, and therapeutic information. According to Goodman—

the sphygmomanometer is now as much a part of the physician's proper armamentarium as are the clinical thermometer and the stethoscope, and the assistance which may be expected from its routine employment should also be as familiar to him.

While the blood-pressure picture has been worked out to some degree of satisfaction by several investigators in Europe and in America, very little work has been done on the subject in the Philippine Islands. The only observations that can be found on record are those of Musgrave and Sison(1) and of Chamberlain.(2) These investigators discuss only the systolic pressure and give no data on diastolic and pulse pressures. Only very recently Warfield(3) pointed out the importance of the determination of diastolic and pulse pressures, a fact that has been neglected or given very little attention in the last few years. According to Warfield blood-pressure estimation should register three values—namely, the height of the systolic; the height of the diastolic; and the difference between the two, the pulse pressure. A record containing the three values he called "blood-pressure picture." Any report which does not register these values he considered as incomplete, and it may lead one to fallacious conclusions.

The systolic pressure is the measure of the maximum force of the heart beat. It is the force necessary to overcome the peripheral resistance plus the force actually expended in driving the blood through the body.(3) It also represents the maximum kinetic energy of the blood.(4)

The importance of the diastolic blood-pressure determination was also made evident by Emerson(5) as being the measure of

<sup>1</sup> Received for publication May 31, 1916.

the load the vascular system carries, and he regarded it as the true measure of the arterial tension. It is also the most constant of the two pressures, for it does not respond so readily to stimuli of various kinds. It further indicates the load the arteries have all the time to carry and the resistance the heart has to overcome as it begins its ventricular systole.

The significance of pulse pressure, according to Martin,<sup>(4)</sup> is the intermittent burden of pressure imposed on the arteries by the energy of the heart in systole in order to force the blood toward the periphery and maintain the circulation. In other words, it is the amount of pressure exerted by the heart during systole in excess of the diastolic pressure. It measures the dynamic over the potential energy, the systolic pressure being the kinetic energy and the diastolic pressure the potential energy. The systolic and pulse pressures represent myocardial values, while the diastolic represents arterial resistance.

High blood pressure is a compensatory process and represents the attempt on the part of the heart to maintain the circulation in equilibrium against conditions that tend to raise the general peripheral resistance.<sup>(3)</sup>

High systolic and comparatively low diastolic pressures, making an excessively large pulse pressure, may mean functional derangement, aortic regurgitation with compensation, myocardial degeneration without nephritis or arteriosclerosis, or vasomotor derangement from any cause.<sup>(4)</sup>

A high diastolic pressure invariably means constantly increased work on the part of the heart, leading to hypertrophy of the left ventricle. It is also of great practical importance to remember that a constantly high diastolic pressure entails a much greater strain on the vessel walls than the transient systolic pressure and should be taken as a danger signal of probable rupture.

The main object of this investigation is to study the normal blood-pressure picture of Filipinos of all ages and of both sexes in order to establish a standard, which we believe will be very valuable to clinicians and to physicians connected with life-insurance companies as a guide in their daily determination of blood pressure. We have also compared our results with the findings of investigators in temperate climates.

#### NUMBER AND CLASS OF PERSONS EXAMINED

We present here the results of blood-pressure readings on 697 males and 218 females, making a total of 915 cases. These observations, with the exception of 78 cases, were made on



Filipinos between the ages of 15 and 87 for males and between 16 and 45 for females. Of 697 male subjects examined, 585 were convicts in Bilibid Prison and 117 were medical students, nurses, or instructors in the College of Medicine and Surgery. Of the 218 females, 128 were convicts and only 90 were nurses and medical students.

TABLE I.—*Summary of cases examined for blood pressure.*

Persons examined.	Males.	Females.	Total.
Convicts in Bilibid Prison .....	585	128	713
Medical students, nurses, and instructors .....	112	90	202
Total .....	697	218	915
Cases used in this report .....	586	181	717
Cases not read .....	67	86	103
Cases apparently sick .....	9		9
Opiate addicts and strangers .....	77	1	78
Miscellaneous .....	8		8
Total .....			915

#### BLOOD-PRESSURE APPARATUS AND PROCEDURE

The apparatus used for all of these determinations was the original Erlanger<sup>(6)</sup> sphygmomanometer; to this we attached a signal magnet to mark exactly the fall of pressure in the mercury column to facilitate the reading.

All the determinations were made on the right arm of the subject, who was seated so that the cuff of the sphygmomanometer was approximately on the level of the heart to eliminate the effect of hydrostatic pressure. After the signal-magnet lever and the sphygmomanometer lever were put in vertical alignment, the pressure was raised until the radial pulse was no longer perceptible. Pressure was generally raised well above the maximum and then lowered by the continuous escapement method.<sup>(6)</sup> We used this method in all our observations for two reasons: it is less troublesome and less painful to the subject and it is quicker than, and just as accurate as, the intermittent-escapement method. The appearance of the radial pulse was noted, and in this way a comparative record of the systolic pressure was obtained—one shown by the appearance of the pulse, and the other by the tracing on the apparatus. We felt the pulse at a pressure of from 1 to 15 or even 20 millimeters of mercury lower than the systolic pressure as indicated by the graphic method.

We endeavored to reduce to a minimum all factors that might affect blood pressure, such as emotions, excitement, ingestion of food, exercise, and contractions of the muscles under the cuff, by explaining to each subject the object of the work, by giving him at least five minutes rest before his blood pressure was taken, and by making these determinations in the majority of cases in the hours between meals—that is, between 9 and 11 o'clock in the morning.

In all of our observations a cuff 10.5 centimeters wide was used with the exception of the first sixty cases, in which a 12.5-centimeter cuff was employed. By comparing the readings taken with the two cuffs, we found an average difference of 2.4 millimeters in the systolic reading and 0.8 millimeter in the diastolic. In all the tables that follow, the readings have all been reduced to the basis of the 12.5-centimeter armlet.

#### CRITERIA USED IN READING SYSTOLIC AND DIASTOLIC BLOOD PRESSURE

The criteria we used in reading the systolic pressure are the same as those used by Erlanger,<sup>(7)</sup> as follows: *a*, a marked increase in the amplitude of the pulsation traced on the revolving drum (Plate II, fig. 2); *b*, a change in the direction in the trough line (Plate II, fig. 1); *c*, a marked separation of the limbs of the pulse wave (Plate II, fig. 1).

The criterion used in determining diastolic pressure is the marked decrease in amplitude in the oscillations (Plate II, fig. 2).

In determining systolic pressure, we gave more attention to criteria *a* and *c* and least attention to criterion *b*.

#### DIFFICULTIES MET IN THE METHOD USED IN READING THE SYSTOLIC PRESSURE

In reading our records, we often met such difficulties as the noncoincidence of the two criteria, that is, when the marked increase in amplitude of the oscillation is not coincident with the marked separation of the limbs of the pulse wave (Plate II, fig. 3, and Plate III, fig. 1). In these cases we generally base our reading on the criterion that appears first.

In a few cases criteria *a* and *c* appeared, disappeared, then appeared again (Plate III, fig. 3). This phenomenon is due to respiration as shown by Weyssse and Lutz.<sup>(8)</sup> In these cases we read at the criterion nearest to the appearance of the pulse as felt on the radial artery.

Infrequently we found subjects who, during the course of the observation, moved their arms and thus caused a marked in-

crease in amplitude in the record of the pulse wave. Such a variation may be very hard to differentiate from criterion *a*. Sometimes movements of the subjects may be due to a distinct shock, as pointed out by Erlanger.(6) The shock is explained by him as follows:

that as soon as the external pressure falls below the maximum intravascular pressure the artery opens out for a moment with the advent of the pulse wave, only to collapse with a perceptible shock almost immediately.

In fact, v. Frey(9) used this shock as an index in his method of estimating systolic blood pressure in man. To overcome this difficulty, we usually repeat our observation.

In the cases where none of the criteria is present, we do not give any reading.

#### RESULTS

Table II shows the averages of our readings arranged in decades, excepting the cases between 15 and 20 years of age.

It will be seen that there is a general tendency for the systolic, diastolic, and pulse pressures to ascend with age. This can be more easily seen in the chart (fig. 1). It will further be seen that with an average age in males of 29.5 years and in females of 25.1 years our cases show average systolic pressures of 115.5 and 116 millimeters of mercury, diastolic pressures of 79.1 and 83.4 millimeters, and pulse pressures of 36.4 and 32.6 millimeters, respectively.

TABLE II.—*Blood-pressure and pulse-rate readings on 536 male and 181 female Filipinos.*

#### MALES.

Age.	Cases.	Systolic pressure.	Diastolic pressure.	Pulse pressure.	Pulse rate.
		<i>mm. Hg.</i>	<i>mm. Hg.</i>	<i>mm. Hg.</i>	
15 to 20 years.....	114	110.7	77.6	33.1	78.2
21 to 30 years.....	258	111.9	76.6	35.3	84.9
31 to 40 years.....	74	118.8	80.3	38.5	80.2
41 to 50 years.....	45	125.4	86.6	38.8	69.0
51 to 60 years.....	24	129.0	86.2	42.8	69.0
61 to 90 years.....	21	137.8	91.6	46.2	77.5
Total and average .....	536	115.5	79.1	36.4	81.5

#### FEMALES.

15 to 20 years.....	66	109.2	80.8	28.4	86.3
21 to 30 years.....	85	118.1	84.1	34.0	80.7
31 to 40 years.....	18	118.2	85.2	33.0	91.4
41 to 60 years.....	12	132.9	90.5	42.4	84.1
Total and average .....	181	116.0	83.4	32.6	87.2

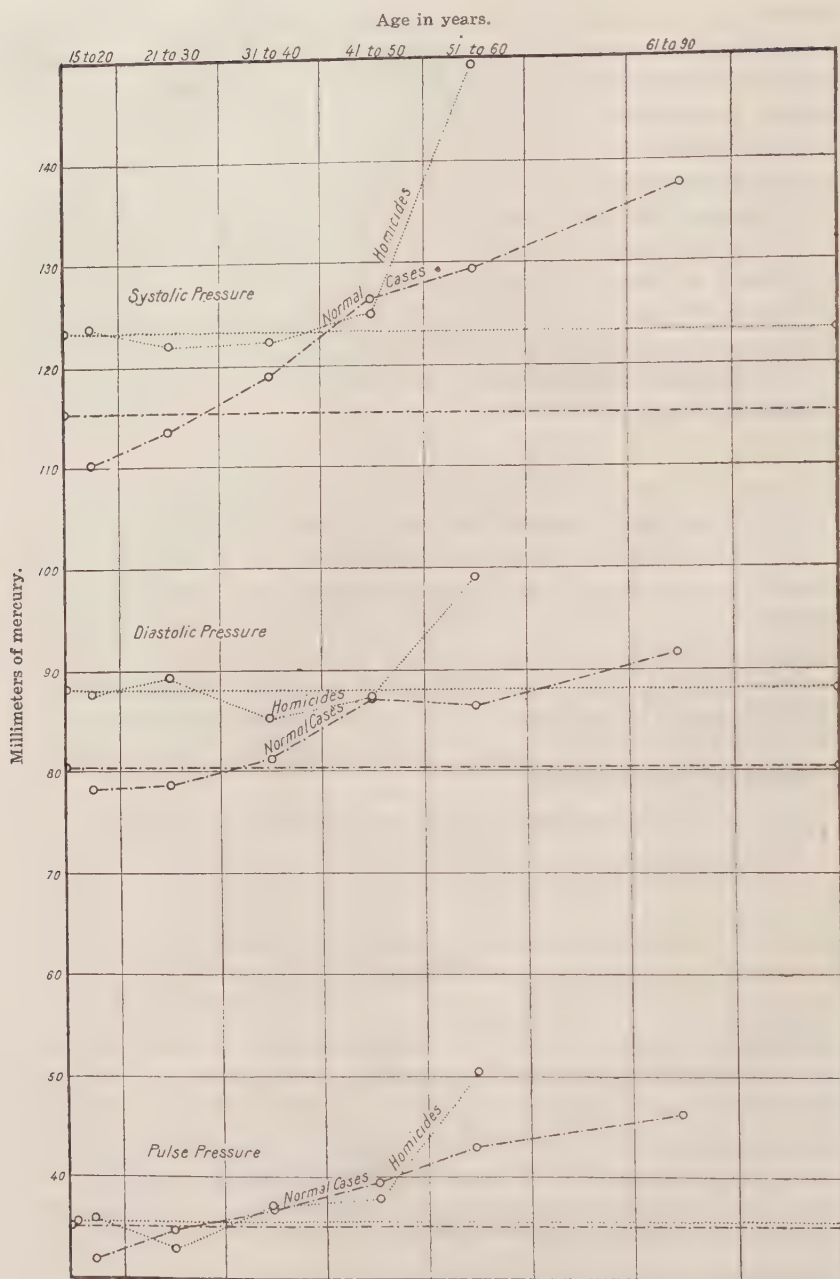


FIG. 1. Normal blood pressure and blood pressure of homicides.

Our results show higher blood-pressure readings with the females than with the males. We account for these unexpected results chiefly by two facts: (a) Of the 546 males, 344, or 64



per cent, were convicts newly admitted to Bilibid Prison; they were quarantined for five days and had no work to do for from one to five days before their blood-pressure readings were taken; whereas all the female convicts were engaged in embroidery work and most of the female nurses were just from the wards when their blood pressures were determined; (b) we have found it very much harder to eliminate the psychic factor with the females than with the males. Although we have in almost all cases discarded the first readings in nervous, excited females, yet we feel we have not been able to eliminate this psychic factor entirely.

Musgrave and Sison(1) in a series of nineteen females, average age not stated, obtained an average systolic pressure of 113 millimeters of mercury, which is 3 millimeters lower than our finding. We can ascribe this difference from our results to the greater number of cases which we have observed, not to mention the differences in age and the conditions to which the subjects were subjected during examination.

An analysis of the first 372 cases shows that in 14.4 per cent the systolic pressure is below 100 millimeters of mercury, in 9.2 per cent it is above 140 millimeters, thus leaving 76.4 per cent with a systolic pressure ranging from 100 to 140 millimeters of mercury. If we take all those cases within 10 millimeters of the average systolic pressure for a given age, we shall notice how very variable the systolic pressure is for that age. Thus, in those cases whose ages range from 15 to 20 years, only 55.4 per cent show a systolic pressure within 10 millimeters of mercury of the average obtained for that age. If we make the same calculation with the diastolic pressure, we shall find similar variations, but to a much less extent, as shown in Table III.

TABLE III.—*Cases with blood pressure within 10 per cent of the average pressure for the given age group.*

Age.	Cases within 10 millimeters mercury of the average.	
	Systolic pressure.	Diastolic pressure.
Years.	Per cent.	Per cent.
15 to 20 .....	55.4	78.4
21 to 30 .....	64.0	74.3
31 to 40 .....	37.7	57.4
41 to 50 .....	44.4	58.4
51 to 60 .....	54.4	68.2

It is interesting to find that out of the twenty-one cases whose ages range from 51 to 87 years eight had a systolic pressure below 150 millimeters and five a pressure below 130 millimeters. We were surprised to find that our oldest subject, an apparently healthy old man of 87 years, had a systolic pressure of 124.6 millimeters of mercury.

TABLE IV.—*Comparison of systolic-pressure readings obtained by the authors with those given by Chamberlain and by Woley.*

Chamberlain with Filipino subjects:

Apparatus	Riva Rocci
Cuff (centimeters)	12.5
Cases	366
Average age (years)	25
Average systolic pressure (millimeters of mercury)	116
Systolic blood pressure (millimeters of mercury)—	
15 to 20 years of age	112.8
21 to 25 years of age	115.4
26 to 30 years of age	117
31 to 35 years of age	116.9
36 to 40 years of age	112.3
41 to 50 years of age	114

Concepcion and Bulatao with Filipino subjects:

Apparatus	Erlanger
Cuff (centimeters)	12.5
Cases	717
Average age (years)	28.5
Average systolic pressure (millimeters of mercury)	115.6
Systolic blood pressure (millimeters of mercury)—	
15 to 20 years of age	110.1
21 to 30 years of age	113.5
31 to 40 years of age	118.9
41 to 50 years of age	126.6
51 to 60 years of age	129.6
61 to 90 years of age	137.8

Woley with American subjects:

Apparatus	Tycos
Cuff (centimeters)	12.5
Cases	1,000
Average age (years)	(?)
Average systolic pressure (millimeters of mercury)	127.5
Systolic blood pressure (millimeters of mercury)—	
21 to 30 years of age	122
31 to 40 years of age	127
41 to 50 years of age	130
51 to 60 years of age	132

Table IV is intended to show a comparison of our systolic readings with those of Chamberlain's<sup>(2)</sup> work on Filipinos and Woley's<sup>(11)</sup> on Americans in the United States. It is evident

that we have obtained practically the same systolic pressure as did Chamberlain on Filipinos. However, we should expect a higher average systolic pressure because the average age of our cases is slightly higher than those of Chamberlain, and because we have used the graphic Erlanger method which is generally known to give higher readings than does the palpatory method (Erlanger gives 1 to 10 millimeters higher). That we did not obtain this result may be explained by the fact that, whereas Chamberlain's cases were "constantly engaged in vigorous out-of-door work, with no special effort taken to protect them from the sunlight," a majority of our male cases (about 64 per cent) had been doing nothing for from one to five days previous to the determinations.

The systolic pressures of the Filipinos are very much lower than those of the Americans living in temperate climates (Woley, Table II), but the same as those of the Americans living in the tropics (Chamberlain) whose ages range between 18 and 50, the average being 26.6 years. These findings suggest that in the tropics systolic pressures are very much lower than in temperate climates.

Bing,<sup>(12)</sup> after an examination of 298 cases, concludes that "the normal systolic blood pressure seems to be 100 to 130 millimeters mercury but seldom with this range above 60 years of age." Earlier determinations of systolic pressure on man were very much exaggerated because narrow cuffs were used.

Goodman<sup>(15)</sup> includes under hypertension all cases with a systolic pressure above 150 millimeters of mercury and a diastolic pressure above 90 millimeters of mercury.

We have been unable to find data of diastolic pressure for man taken with the Erlanger sphygmomanometer, except a series of determinations by Erlanger upon himself in which he obtained an average of 80.5 millimeters of mercury using a 13-centimeter cuff. Most of the limited data given for diastolic pressure are read by the auscultatory and the oscillatory methods.

However, we do not intend to go into these methods in detail. It suffices to mention that there are great differences between data given by different persons. Weyssse and Lutz,<sup>(8)</sup> using the auscultatory method in a series of ten students, obtained an average of 85 millimeters. With a similar method on a series of young adults McWilliam and Melvin<sup>(13)</sup> gave 65 millimeters of mercury; Bachmann,<sup>(14)</sup> using Pachon's sphygmomanome-

tric oscillometer on eighty-five subjects, gives the following results:

TABLE V.—*Blood pressure recorded by Bachmann on eighty-five subjects.*

Age in years.	Millimeters of mercury.
15 to 20	95.5
21 to 30	94.0
31 to 40	88.7
41 to 50	89.4
51 to 60	90.0
61 to 70	85.0

These findings showed that diastolic-pressure readings in man vary not only according to the instrument used but also with age. As seen in Table I, our findings showed that the average diastolic pressure for each decade varies from 76.6 to 91.6 millimeters in males and between 80.8 to 90.5 millimeters in females.

#### DISCUSSION

Our findings show conclusively that systolic pressures of Filipinos are very much lower than those of Americans (Woley(11)) and Europeans (Potain(16)). If we take into consideration that our observations were made on the Erlanger sphygmomanometer, which gives comparatively higher readings (1 to 10 millimeters or even 20 millimeters as found by the appearance of the radial pulse in our cases), the difference is much greater.

Huggard(17) claims that this low blood pressure found in the tropics is associated with the dilation of the peripheral blood vessels as well as increased elasticity of the vessel walls. Musgrave and Sison(1) believe that it is due, first, to low peripheral resistance associated with the increased secretory function of the sweat glands and, secondly, to the possibility of splanchnic influences associated with prevalent sensation of abdominal vacuity and to the frequency of gastrointestinal disturbances. Chamberlain suggests that there is a vaso-constriction of the superficial blood vessels in the tropics. This will not only explain the occurrence of tropical pallor, but will also account for the fact of its disappearance in a few days on the return of the subject to a cold region.

#### PULSE RATE

It is a known physiological fact that pulse rate is influenced by the size of the body, sex, muscular movements, hot food, rise of



temperature, and psychic condition. It decreases from birth up to 20 years. From 20 years onward it remains practically constant; in old age there may be a slight increase (Schäfer(19)).

Pulse rate in the tropics is still an unsettled question. Rat-tray on one hand claims that it is lower in the tropics; Huggard, Parks, Jousset, and Chamberlain maintain, on the contrary, that the pulse rate is increased by ten beats a minute. Chamberlain,(2) in a series of 200 Filipinos with an average age of 24.9 years, found an average of 79.1 beats a minute. Our 536 male cases, of an average age of 29.5 years, gave an average pulse rate of 81.5 beats a minute; and in 181 female cases, average age 25.1 years, we obtained an average of 84.1 beats a minute. These results compare favorably with Chamberlain's findings. Table I shows that the pulse rate has no definite relation to age nor to blood pressure. As is to be expected, the pulse rate in females is a few beats more than in males. Our findings, then, confirm the results of Huggard and others.

One more interesting thing we found in our male cases was that after 50 years of age the pulse rate begins to fall slightly. Table I shows that 69 cases between 41 to 60 years of age gave an average of 69 pulse beats in a minute. After 60 years it begins to increase again slightly. Our 21 cases between 61 to 90 years of age gave 77.5 beats a minute. Stewart(21) gives an average of 73 to 69 beats a minute from 20 to 25 years. According to this author it remains the same until the age of 60 and then increases again somewhat until old age.

No deductions can be drawn as to the relation of pulse rate and the height of arterial tension, since the form of each pulse curve depends not only upon the systole of the heart, but also on the peripheral resistance, the tone of the arteries, and the amplitude of the reflected waves.

#### RELATION OF SYSTOLIC PRESSURE TO CRIME

During this investigation we thought that it would be interesting to find out if we can establish any relation between systolic pressure and crime. The result of this inquiry gave us the following findings: Thirty-three cases in our series were homicides with an average age of 30.6 years. These had an average systolic pressure of 123.5 millimeters, 88.1 millimeters diastolic, and a pulse rate of 90.2 per minute; five other cases were excluded on account of the systolic pressure being above 160 millimeters. The figures for these homicides are higher than normal with the exception of five cases between 41 and 50 years

in whom the systolic pressure is 1.6 millimeters lower than normal.

The personal histories of these cases are negative in regard to illegitimacy and mental disturbance, past or present, with the exception of two who gave histories of epilepsy, three of alcoholic habit in the parents, one of alcoholic habit, and three histories of injury on the head. The Wassermann test was not performed in any one of them, so the syphilitic factor cannot be ruled out. We hesitate to offer any explanation on this strange finding on account of the limited number of cases studied.

Castellani and Chalmers(17) suggested that tropical heat, together with the effect of the actinic rays of the sun, may result in weakening of the control of the higher centers over the lower and thus induce outbursts of what Plehn called "tropical fury," by which he means fits of passion caused by trivial incidents. According to Castellani and Chalmers this is one of the causes of assault and violent crimes in certain parts of the tropics.

TABLE VI.—*Blood pressures and pulse rate of homicides.*

[Average age, 30.6 years.]

Age.	Cases.	Systolic pressure. <sup>a</sup>	Diastolic pressure.	Pulse pressure.	Pulse rate.
		<i>mm. Hg.</i>	<i>mm. Hg.</i>	<i>mm. Hg.</i>	
15 to 20 years -----	4	123.8	87.9	35.9	99
21 to 30 years -----	16	122.0	89.1	32.9	99.2
31 to 40 years -----	7	122.3	85.2	37.1	85.7
41 to 50 years -----	5	125.0	87.2	37.8	83.2
51 to 60 years -----	1	149.6	99.2	50.4	90
Total and average -----	33	123.5	88.1	35.4	90.2

<sup>a</sup> Five cases with systolic pressure above 166 millimeters are excluded.

#### BLOOD PRESSURE IN OPIUM AND MORPHINE HABITUÉS

Petty,(20) in his investigation in morphine habitués, uniformly found high blood pressures on admission. In our series of 915 cases we have 65 cases that are opium and morphine addicts. Their blood pressures were taken between the second and fifth day after they were admitted to the prison. It is very interesting to find that all of them, with the exception of 14 cases who had been taking the drug for periods of from ten to twenty years (Table VII), showed a very much lower systolic pressure than our normal cases of the same age. Our 65 cases with an average age of 40.6 years gave an average of 107.6 millimeters

systolic, 76.8 millimeters diastolic, 30.8 millimeters pulse pressure, and 79.7 pulse rate per minute.

It is also surprising to find that four cases of an average age of 44 years who had taken the drug for twenty or more years showed an average of 97.8 millimeters of mercury systolic pressure, 71 millimeters diastolic, and 25.9 millimeters pulse pressure.

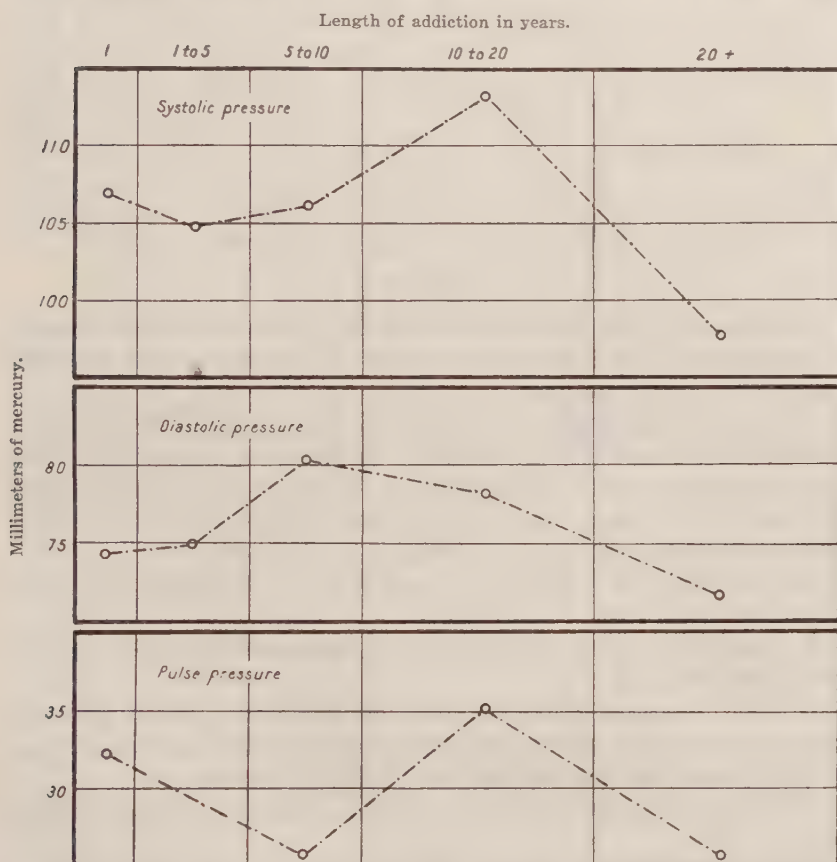


FIG. 2. Blood-pressure pictures of opium and morphine addicts arranged according to length of time the drug has been taken.

sure. If we compare these results with our normal cases, we find that between 41 and 50 years we have an average of 126.6 millimeters systolic, 87.1 millimeters diastolic, and 39.5 millimeters pulse pressure. These findings, then, appear to be contrary to the finding of Pettey. While the systolic pressure increases with age in normal individuals, morphine and opium addicts may have a very low blood pressure as the results of the long-continued use of the drug (fig. 2).

TABLE VII.—*Blood pressures and pulse rates of opiate addicts arranged according to lengths of time opium or morphine has been taken.*

Drug habit.	Cases.	Average age.	Systolic pressure.	Diastolic pressure.	Pulse pressure.	Pulse rate.
		Years.	mm. Hg.	mm. Hg.	mm. Hg.	
Less than 1 year -----	6	41.3	106.9	74.5	32.4	74.3
1 to 5 years -----	18	40.5	104.7	75.0	29.7	82.1
5 to 10 years -----	11	40.0	106.3	80.4	25.9	89.1
10 to 20 years -----	14	0.6	113.4	78.2	35.2	73.6
Over 20 years -----	4	44.0	97.8	71.9	25.9	71.0
(?) -----	12	39.9	110.3	77.2	33.1	<sup>a</sup> 78.8
Total and average -----	65	40.6	107.6	76.8	30.8	<sup>b</sup> 79.6
Range (cuff 10.5) -----		66-21	142-80	100-59	59-12	130-59

<sup>a</sup> Five cases out of the 12.<sup>b</sup> Fifty-eight cases out of the 65.

## SUMMARY

By using the graphic method with the Erlanger sphygmomanometer and a 12.5-centimeter cuff, our 717 cases with an average age of 28.5 years showed:

1. An average systolic pressure of 115.5 millimeters of mercury in males and 116 millimeters in females, an average diastolic pressure of 79.1 millimeters in males and 83.4 millimeters in females, and hence an average pulse pressure of 36.4 millimeters in males and 32.6 millimeters in females.

2. That there is a general rise of systolic, diastolic, and pulse pressures with age.

3. That for a given age the systolic pressure is more variable than the diastolic.

4. That the pulse pressure does not differ very much between the different ages; that cases with constant pulse pressure below 20 and above 50 millimeters are probably abnormal.

5. That hypertension is not a necessary accompaniment of old age.

6. That the pulse rate of Filipinos averaged a few beats above the usual standard of 72 per minute.

7. That in thirty-three homicides the systolic and diastolic pressures were, respectively, 7.9 and 7.8 millimeters higher than normal, while the pulse pressure remained the same.

8. That in sixty-five opium and morphine habitués all the blood-pressure averages are lower than normal.

In conclusion we wish to express our obligation to Doctors Mañalak and Smith, of Bilibid Prison, and to Miss Dobbs, of the Philippine Training School for Nurses, for their valuable assistance in securing subjects for us.



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## ILLUSTRATIONS

### PLATE I

The sphygmomanometer.

### PLATE II

- FIG. 1. Record 184. Coincidence of the three criteria used in reading systolic pressure.
2. Records 717 and 720. The first crosses mark the points where the systolic pressure is read—sudden increase in amplitude of oscillations. The second crosses mark the points where diastolic pressure is read.
  3. Record 190. The cross marks the point where there is a marked increase in amplitude of oscillation followed by marked separation of the limbs in the next pulse wave.

### PLATE III

- FIG. 1. Record 259. Appearance of separation of the limbs ahead of the marked increase in amplitude.
2. Two records where the systolic pressure cannot be read.
  3. Records 533 and 648. Each of criteria (a) and (c) appear more than once.

### TEXT FIGURES

- FIG. 1. Normal blood pressure and blood pressure in homicides.
2. Blood-pressure picture of opium and morphine addicts arranged according to lengths of time the drug has been taken.





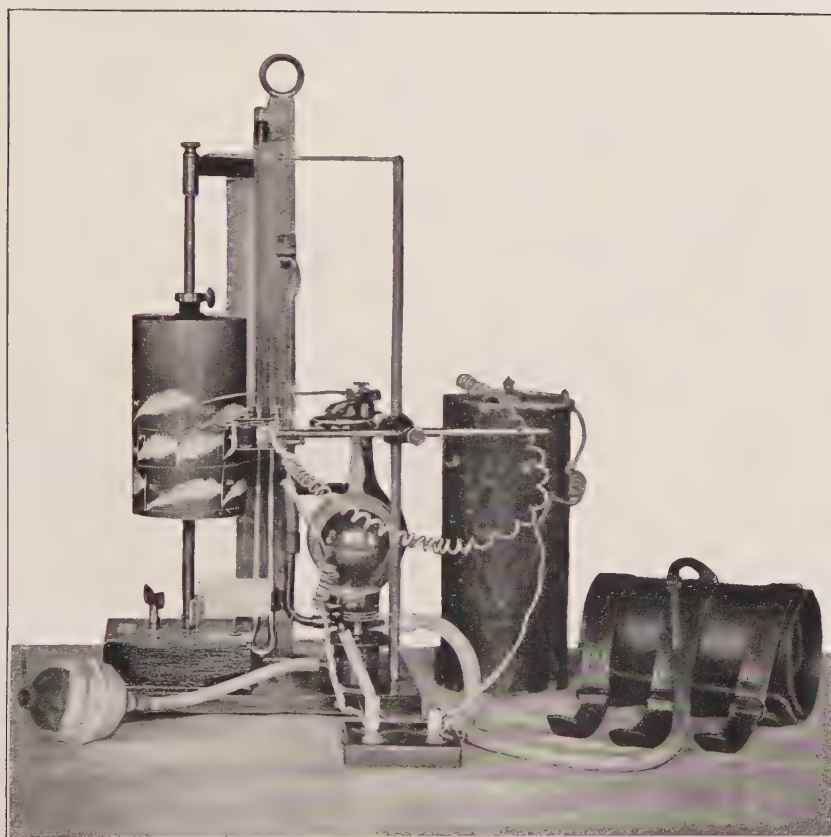


PLATE I. THE SPHYGMOMANOMETER.



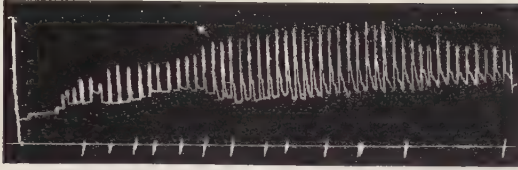


Fig. 1. Record 184. Coincidence of the three criteria used in reading systolic pressure.

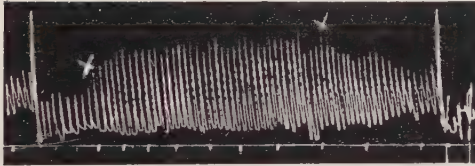


Fig. 2. Records 717 and 720. The first crosses mark the points where the systolic pressure is read—sudden increase in amplitude of oscillations. The second crosses mark the points where diastolic pressure is read.



Fig. 3. Record 190. The cross marks the point where there is a marked increase in amplitude of oscillation followed by marked separation of the limbs in the next pulse wave.

PLATE II.





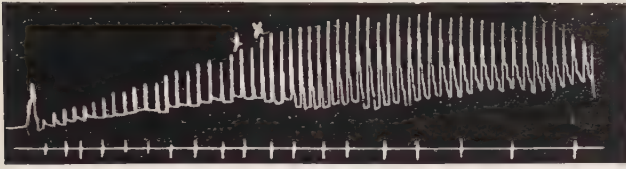


Fig. 1. Record 259. Appearance of separation of the limbs ahead of the marked increase in amplitude.

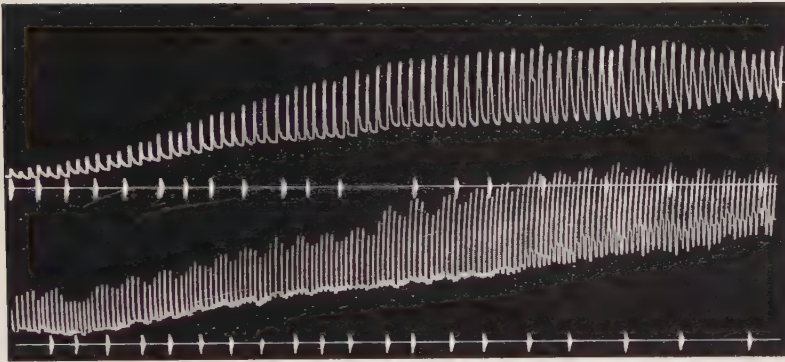


Fig. 2. Two records where the systolic pressure cannot be read.

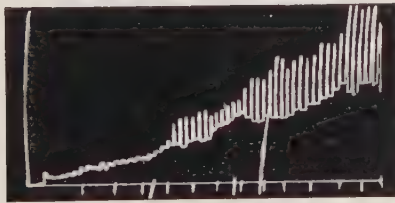


Fig. 3. Records 533 and 648. Each of criteria (a) and (c) appearing more than once.



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### THE RELATION BETWEEN THE AMOUNT OF CHOLERA CULTURE INJECTED INTO THE GALL BLADDER AND THE STATE OF CHOLERA CARRIERS IN EXPERIMENTAL ANIMALS

By OTTO SCHÖBL

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

In the work on experimental cholera carriers the following question naturally presented itself: What relation exists between the amount of cholera vibrios injected into the gall bladder and the state of cholera carriers in experimental animals? In previous experiments<sup>1</sup> the amount injected was considerable—that is, one tenth of one slant. Under such conditions all inoculated guinea pigs were positive up to the thirteenth day, inclusive. It remained to be decided whether the number of “takes” would be as high as that, if a smaller amount of cholera culture be injected, and whether or not the duration of the carrier state would be affected by the variation of the amount of cholera injected into the gall bladder. The following experiments were arranged so that amounts of cholera culture varying from 1/80 to 1/100,000,000 of a slant were injected. The animals of one lot were killed<sup>2</sup> on the seventh and those of another lot on the thirteenth day after inoculation.

From these experiments it is evident that the amount of cholera culture injected into the gall bladder can be decreased considerably and still all of the inoculated animals will become carriers. In our experiments the inoculum was decreased from 1/80 to 1/80,000 of one slant. The percentage was just as high as it was when 1/10 of a slant was inoculated. When the inoculum was diluted beyond this limit, the results became irregular. Animals that received 1/100,000 and 1/10,000,000 of a slant, respectively, were found negative, while the animal that was inoculated with 1/100,000,000 of a slant was found positive.

<sup>1</sup> *Journ. Inf. Dis.* (1916), 18, 307-314.

<sup>2</sup> *Op. cit.*

TABLE I.—Showing the number of takes in experimental cholera carriers after intravesicular inoculation of decreasing doses of cholera culture.

[Animals killed seven days after inoculation.]

Dilution.	Direct plates.				Peptone cultures.			
	Gall bladder.	Duode-num.	Ileum.	Cæcum.	Gall bladder.	Duode-num.	Ileum.	Cæcum.
1/80.....	n	f	n	vn	+	+	+	+
1/160.....	vn	f	f	—	+	+	+	—
1/320.....	vn	vf	vf	—	+	+	+	—
1/640.....	vn	f	vf	—	+	+	+	+
1/1,000.....	n	vf	n	f	+	+	+	+
1/2,000.....	n	vf	f	—	+	+	+	—
1/4,000.....	vf	—	—	—	+	+	+	—
1/8,000.....	vf	—	—	—	+	+	+	+
1/20,000.....	n	vf	vf	—	+	+	+	—
1/40,000.....	vf	—	—	—	+	+	+	—
1/80,000.....	n	f	f	—	+	+	+	—
1/100,000.....	—	—	—	—	—	—	—	—
1/10,000,000.....	—	—	—	—	—	—	—	—
1/100,000,000.....	vn	n	vn	f	+	+	+	+

+ = cholera vibrios found; — = cholera vibrios not found; vf = less than half a dozen colonies; f = about one dozen colonies; n = about 200 colonies; vn = more than 200 colonies.

TABLE II.—Showing the duration of carrier state in experimental animals after intravesicular injection of decreasing doses of cholera culture.

[Animals killed thirteen days after inoculation.]

Dilution.	Direct plates.				Peptone cultures.			
	Gall bladder.	Duode-num.	Ileum.	Cæcum.	Gall bladder.	Duode-num.	Ileum.	Cæcum.
1/80.....	f	—	—	—	+	+	+	—
1/160.....	f	—	—	—	+	+	+	—
1/320.....	vf	—	—	—	+	—	+	—
1/640.....	f	—	—	—	+	+	+	—
1/1,000.....	f	vf	f	—	+	+	+	—
1/2,000.....	n	vf	(a)	—	+	+	+	—
1/4,000.....	f	—	—	—	+	+	+	—
1/8,000.....	n	f	f	—	+	+	+	+
1/20,000.....	vf	—	—	—	+	+	+	—
1/40,000.....	—	—	—	—	—	—	—	—
1/100,000.....	—	—	—	—	—	—	—	—
1/100,000,000.....	—	—	—	—	+	+	+	+

<sup>a</sup> Vibrios present, but overgrown by other bacteria.

Table II shows the duration of carrier state in animals inoculated with decreasing amounts of cholera culture. All of the carriers that were infected with from 1/80 to 1/20,000 of a slant lasted the usual length of time, which was found in our



previous experiments<sup>3</sup> to be thirteen days. Animals injected with 1/40,000 and 1/100,000 of a slant were found negative. Again the animal that received the smallest dose, 1/100,000,000, still harbored cholera vibrios on the thirteenth day.

#### CONCLUSIONS

1. Inoculation of a relatively small amount of cholera vibrios into the gall bladder may produce carriers in animals, but the results are not as regular as they are when larger amounts are used for inoculation.

2. The amount of inoculum seems not to have any direct bearing on the duration of the carrier state in animals according to the preceding experiments. The negative animals which were inoculated with 1/40,000 and 1/100,000 are to be interpreted as failure "takes," as the animal that received a far smaller dose was positive.

<sup>3</sup> *Op. cit.*



# THE INFLUENCE OF BILE UPON THE DURATION OF THE STATE OF CHOLERA CARRIERS IN EXPERIMENTAL ANIMALS

By OTTO SCHÖBL

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

In order to gain some information with regard to the relation of cholagogs to the state of cholera carriers, some experiments were arranged in the following way.

Thirteen guinea pigs of the same size were inoculated simultaneously. One tenth of one slant of cholera culture was injected into the gall bladder.<sup>1</sup> After the inoculation seven of the animals were given 1 cubic centimeter of ox bile by mouth every third day. They were killed and examined at intervals as indicated in Table I. The remainder of the experimental carriers, six in number, which received no bile, were taken as controls.

TABLE I.—Showing the influence of bile upon the duration of the state of cholera carriers in experimental animals.

## TREATED ANIMALS.

Killed after inoculation.	Direct plates.				Peptone cultures.			
	Bile.	Duoden-um.	Ileum.	Cæcum.	Gall bladder.	Duoden-um.	Ileum.	Cæcum.
<i>Days.</i>								
16.....	n	—	f	—	+	—	+	—
16.....	n	—	f	—	+	—	+	+
19.....	n	f	f	—	+	+	+	+
19.....	vn	vf	n	—	+	+	+	+
21.....	n	—	n	—	+	—	+	—
28.....	n	—	n	—	+	—	+	—
35.....	n	f	f	—	+	+	+	+

## UNTREATED CONTROLS.

<i>Days.</i>								
16.....	n	—	vf	—	+	+	+	+
16.....	—	—	—	—	—	—	—	—
19.....	n	—	f	—	+	+	+	+
21.....	—	—	—	—	—	—	—	—
28.....	f	—	—	—	+	—	—	—
35.....	—	—	—	—	—	—	—	—

+ = cholera vibrios found; — = cholera vibrios not found; vf = less than half a dozen colonies; f = about one dozen colonies; n = about 200 colonies; vn = more than 200 colonies.

<sup>1</sup> For technique, see *Journ. Inf. Dis.* (1916), 18, 307–314.

Seven of the seven experimental cholera carriers fed on bile were found positive from sixteen to thirty-five days after the inoculation, while only three of the six control carriers were found to harbor cholera vibrios during the same period of time.

These experiments show clearly that the increased flow of bile does not further the disappearance of the cholera vibrios from the gall passages and from the intestine. On the contrary, it seems strongly to indicate that the administration of bile, a cholagog "par excellence," tends to prolong rather than to shorten the duration of the state of cholera carrier.



CARBOHYDRATE FERMENTATION BY *BACILLUS PESTIS*, COM-  
PARING CERTAIN AMERICAN AND ORIENTAL STRAINS  
WITH ANALYSIS OF DISCREPANCIES OF FERMENTATIONS WITH HISS'S SERUM  
WATER, LITMUS AGAR, AND BOUILLON <sup>1</sup>

By H. WINDSOR WADE

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

In spite of the great amount of study to which *Bacillus pestis* has been subjected, it cannot be determined positively by a survey of the literature what ability this organism has for breaking down carbohydrates with the production of acid, nor is it clear whether all strains are alike in this regard.

Recently the opportunity has arisen to compare cultures isolated in New Orleans, Louisiana, in 1914, and which at the time were thought possibly to be different from those from the Orient, with a number of strains isolated at different times in the Philippine Islands. As a result of a somewhat intensive study, which was extended considerably beyond the scope at first intended, the fermentative powers of these strains have been clearly defined. The results are recorded, not because of any controversy on the subject, but to establish clearly these features of the biology of *Bacillus pestis* and to emphasize the unusual similarity of the strains studied, as well as to illustrate certain features of fermentation reactions in general.

LITERATURE

Few of the many articles touching on the bacteriology of plague deal with the reactions of the organism in carbohydrate media. The earlier writers knew that acid was produced in dextrose media, and in 1898 Gioso and Biginelli(1) determined this to be lactic acid. Rees(2) said that, according to Klein, *B. pestis* formed a small amount of acid in sugar agar and caused coagulation of milk in about two weeks. Other authors, however, stated that litmus milk was little affected. The German Plague Commission (1897-1898), according to Dieudonné and Otto,(3) tested for gas formation in bouillons containing dextrose, levulose, lactose, and mannite, with negative results. Simple acid formation

<sup>1</sup> Received for publication August 25, 1916.

was not considered an important feature of bacterial biology at that period. The Indian Plague Commission found, according to the same authors, that acid was formed in dextrose, levulose, mannite, and galactose, but not in lactose and dulcitol. Calvert (4) spoke of reddening of litmus glucose agar. Herzog (5) made the statement that it does not ferment dextrose, levulose, lactose, or mannite. He did not say whether this was the result of his own observations nor whether the term "ferment" was intended to signify gas production. It is improbable that he tested for acidification in these media.

The first extended tests were those of MacConkey (6) in 1905. In his bile salt medium for a base he obtained acid without gas in dextrose, levulose, maltose, galactose, mannite, and dextrin, but no change in raffinose, lactose, saccharose, dulcitol, and sorbitol. It is not stated how this acidity was determined. Wherry (7) described the organism isolated from a plague rat as growing only in the open end of fermentation tubes containing 1 per cent dextrose, levulose, lactose, saccharose, and starch bouillons. These cultivations were intended to rule out *B. coli*, and no mention of acid formation was made. Later, however, (8) he described strains from human cases and from ground squirrels, all of which produced acid but no gas in 1 per cent dextrose, levulose, galactose, maltose, and mannite broths, but no change in lactose, saccharose, or inulin. Vourland (9) tested a single strain and found that beside the sugars given by MacConkey glycerin, arabinose, xylose, salicin, saccharose, and lactose gave acid reactions. Dextrin, however, was not acidified, and he noted that saccharose reverted to blue.

MacConkey, (10) in 1908, reiterated his previous results, adding adonite, inulin, amygdalin, and  $\alpha$ -methyl glucoside to his list of unaffected substances, and suggested that Vourland's results might have been due to a possible trace of muscle sugar in the ordinary nutrient agar which he employed. The Advisory Committee (11) confirmed some of MacConkey's reactions, having used his medium with glucose, levulose, mannite, and galactose, which gave acid, and lactose and dulcitol, which were not changed.

McCoy, (11) working in the Hygienic Laboratory in Washington, determined the virulence of old strains from Manila, Bombay, Jedda, New York, Glasgow, San Francisco, and Reedy Island in comparison with newly isolated strains from San Francisco. The old strains had been tested culturally by Wherry, who had found that the activity of the cultures was alike throughout—dextrose, levulose, and galactose being most actively fermented, mannite next, and maltose least. McCoy found no

difference between these and his new strains. Rowland<sup>(13)</sup> described the organism which he employed in vaccine work as forming acid in dextrose and mannite, but not touching lactose, saccharose, dulcitate, adonite, inulin, and litmus milk.

At the International Plague Conference held in Mukden, (1911), the idea that the strain of organism in pneumonic plague differed from that ordinarily found in bubonic plague was discussed. Zabolotny<sup>(14)</sup> inclined to this view, but no definitely confirmatory evidence was adduced. No results of cultivation in sugar media were reported at the conference. In summing up the existing evidence, Strong,<sup>(15)</sup> and later Strong and Teague,<sup>(16)</sup> declared that there was no distinction between the bubonic and the pneumonic organisms demonstrable either culturally or by study of virulence and immunology.

Schöbl,<sup>2</sup> working in the Bureau of Science laboratories in 1914, made parallel reactions on litmus carbohydrate agars, using seventeen reagents and twenty-one strains of *B. pestis*, including known Chinese strains and the laboratory avirulent strain. He demonstrated no qualitative fermentation differences, but obtained results somewhat at variance with those of others in that saccharose and glycerin gave acidification, as well as dextrose, levulose, galactose, maltose, mannite, and salicin. Lactose, raffinose, dulcitate, dextrin, amygdalin, inulin, inosite, adonite, sorbite, and nutrose gave no acid.

The latest report of such an investigation is that of Berlin,<sup>(17)</sup> who studied fifty-five strains, employing fourteen sugars in a concentration of 1.3 per cent in agar. He obtained uniform results, all cultures giving acid in arabinose, glucose, galactose, maltose, mannite, and levulose and no change in saccharose, lactose, raffinose, starch, dextrin, inulin, dulcitate, and adonite. He concluded that the age of the cultures and their virulence played no rôle in the acid formation.

The minor differences which seem apparent upon comparing various reports would appear to be due either to technical variations by different investigators, or to the existence of subspecies recognizable only by special methods of investigation. Analysis of the reports makes the latter hypothesis seem the less probable, since no one has reported variation among the cultures under his hand, no matter how widely his results differed from those of others. There seems to be no evidence of modification of fermentative power after prolonged artificial cultivation.

<sup>2</sup> Personal communication.



## EXPERIMENTAL CULTIVATIONS

Discrepancies among the reports were noticed when, in the summer of 1914, a case of bubonic plague was encountered at the Charity Hospital in New Orleans. The organism from this and the next two cases that appeared were studied within a few days of isolation.<sup>(18)</sup> For comparison, the strain that had been isolated two years previously from a New Orleans rat, at the time of an epidemic in Havana, was tested in parallel. In a few fermentation tests with Hiss's litmus serum-waters dextrose was fermented strongly, galactose weakly, and levulose and maltose, among others, not at all. The only value of these results was the demonstration of similarity between the old rat strain and the newly isolated human strains. The inactivities noted, particularly in view of disagreement among reports, were at the time thought possibly to indicate that the New Orleans strain differed from those encountered elsewhere. It seemed that, could this be established and foreign centers of infection by plague of the same type be located, the original source of the New Orleans invasion might be traced. This possibility made the study of the organisms of rather more than academic interest. An attempt was made to secure other strains for comparative study, but this was not successful, and the subject was not pursued.

The opportunity having now arisen to do so, I have compared, under various conditions, the four New Orleans strains and six strains carried in the biological laboratory of the Bureau of Science. For convenience the organisms have been designated by letters as follows:

- Strain A. From New Orleans rat, 1912.
- B. From New Orleans plague case 1, 1914 (case fatal, organisms killed rats and guinea pigs).
- C. From New Orleans plague case 2, 1914 (case rapidly fatal).
- D. From New Orleans plague case 3, 1914 (case recovered).
- E. From Iloilo, 1912.
- F. Manila case.
- G. Strain "Manila VIII."
- H. Strain "M I."
- I. Strain "M. II."
- J. "Plague avirulent," laboratory strain.

Strains *E*, *F*, and *G* were furnished by Dr. J. A. Johnston; strain *J*, by Dr. O. Schöbl; and strains *H* and *I*, by Mr. A. Guzman; all of the biological laboratory, Bureau of Science. The two last-mentioned strains are said to have been isolated by



Strong in Mukden, though this may be questioned. The Philippine organisms were undoubtedly originally introduced from China. The New Orleans strain *A* was now 4 years old, and strains *B*, *C*, and *D* were 2 years old. It may be noted, as indicating the viability of these cultures, that the transplants made in February, 1916, were from agar cultures, inoculated eighteen months previously, that had been closed with sealing wax and kept at room temperature, which in New Orleans averages rather high. Strain *E* was 4 years old, and the others ranged from 2 to 5 years in age.

#### LITMUS SERUM-WATERS OF HISS

In the first series of cultivation Hiss's serum-water media were used as originally, horse serum being utilized instead of beef serum. The sugars were used in but 0.5 per cent strength on account of the difficulty of obtaining some of them. The total number of reagents used was nineteen. Inulin was not procurable. The results of the positive and irregular reactions in two series appear combined in Table I. For brevity the reagents not showing change by this method are listed in the note to Table I.

The reaction in dextrose was always rapid and complete, and in the mannite cultures coagulation also developed regularly, though considerably more slowly. The levulose cultures reacted with much irregularity. In arabinose there was at times fairly definite acidification, but this was slight and very inconstant in these low-sugar series. Litmus milk usually showed but a faint change. The fourteen media listed as negative, certain of which were tested repeatedly, showed absolutely no change after ten days.

A fact of interest in this series is that there was demonstrated absolutely no distinction between the New Orleans strains and those isolated in the Philippine Islands. This similarity has been found to persist with various media.

These results were so at variance with most authorities, though for the most part comparable with those obtained in New Orleans, that the study was extended to determine the reason for the discrepancies. In order to determine, for one thing, the part that low-sugar concentration played, a small series of 1 per cent media was later inoculated. The reactions obtained with these appear in Table VIII and demonstrate at least a moderate improvement over the 0.5 per cent media.

TABLE I.—Reaction of *B. pestis* in Hiss's litmus serum-waters containing 0.5 per cent carbohydrate.

[In Tables I and VIII the symbols are used as follows (compare with Table X): — = no change; ± = faint acidity, not sufficient to be considered a positive reaction; + = medium reddened, but still clear and fluid; 2+ = medium red and fluid, but with slight cloudiness; 3+ = medium opaque, but fluid; 4+ = medium coagulated. The figures in parentheses indicate the day of cultivation on which the recorded change was observed.]

Strain of organism.	Dextrose.	Mannite.	Levulose. Series II.	Arabinose. Series II. <sup>a</sup>		Litmus milk.
				A.	B.	
A	4+ (2)	+ (1)	+ (1)	2+ (3)	± (3)	±.
		3+ (3)	3+ (3)		2+ (4)	
		4+ (4)	4+ (4)			
B	4+ (2)	+ (3)	Negative	+ (8)	Negative	Negative.
		3+ (4)				
		4+ (6)				
C	4+ (2)	± (2)	do	+ (3)	do	Do.
		3+ (3)		2+ (4)		
		4+ (4)				
D	4+ (2)	± (1)	+ (2)	+ (3)	do	±.
		+ (2)	3+ (3)	2+ (4)		
		3+ (3)	4+ (4)			
E	4+ (2)	4+ (5)				
		± (2)	+ (2)	± (4)	do	±.
		+ (3)	3+ (5)			
F	4+ (2)	3+ (4)				
		4+ (7)				
		+ (1)	+ (3)	+ (4)	do	±.
G	4+ (2)	3+ (4)	3+ (4)			
		4+ (7)				
		+ (1)	+ (4)	+ (3)	do	±.
H	4+ (2)	2+ (3)	3+ (6)			
		3+ (4)				
		4+ (5)				
I	4+ (2)	+ (1)	2+ (3)	+ (3)	± (6)	±.
		2+ (3)	4+ (4)	2+ (4)		
		3+ (4)				
I	4+ (2)	4+ (5)				
		+ (1)	+ (2)	Negative	Negative	±.
		3+ (3)	2+ (4)			
		4+ (5)				

NOTE.—The following sugars were not fermented in these series: Maltose, galactose, salicin, glycerin, saccharose, lactose, dextrin, nutrose, dulcitol, adonite, raffinose, sorbitol, inositol, amygdalin, and arabinose.

<sup>a</sup> Results of successive sets with the same lot of medium.

#### LITMUS-SUGAR AGARS

To control the results obtained with serum waters, a parallel set of cultures was made using litmus-sugar agar slants. In Table II are shown details of reactions in the five media which, of the eighteen used in the primary 0.5 per cent set, were acidified. Later, on account of the results with bouillon titration, a 1 per

cent set was cultivated, using only the weakly or irregularly fermented substances—salicin, arabinose, galactose, maltose, and glycerin. The reactions with these are included in the table. The stages of acidification are approximately indicated in a manner which is of use for purposes of comparison.

TABLE II.—*B. pestis* on litmus-sugar agars (incubation at 37° C. for ten days).

[In Table II the symbols used are as follows: + = slight reddening of agar beneath the growth; 2 + = a spreading area of acidification; 3 + = diffusion of acid not quite complete; 4 + = complete reddening of the entire slant. The figures in parentheses indicate the days after inoculation on which the changes were noted.]

	Series I. 0.5 per cent.					Series II. 1 per cent.				
	Dex- trose.	Man- nite.	Levul- ose.	Malt- ose.	Glyc- erin.	Malt- ose.	Glyc- erin.	Arabin- ose.	Galac- tose.	Salicin.
A -----	4+(2)	4+(2)	4+(4)	4+(2)	—	4+(7)	—	4+(3)	4+(5)	4+(7)
B -----	4+(2)	4+(2)	4+(2)	(a)	—	4+(10)	—	4+(3)	4+(5)	4+(5)
C -----	4+(2)	4+(2)	4+(3)	(a)	—	3+(10)	—	4+(3)	4+(5)	4+(5)
D -----	4+(2)	4+(2)	4+(2)	(a)	—	4+(7)	—	4+(3)	4+(5)	4+(7)
E -----	4+(3)	4+(2)	4+(4)	4+(3)	—	4+(5)	—	4+(5)	2+(10)	4+(5)
F -----	4+(3)	4+(3)	4+(3)	±(1) +(2) 2+(3) +(6) —(7)	—	4+(4)	—	4+(5)	2+(10)	4+(5)
G -----	4+(4)	4+(3)	4+(4)	4+(4)	4+(5)	4+(5)	4+(10)	4+(5)	1+(2) 2+(3) 1+(4) —(5) +(7) 2+(10)	(a)
H -----	4+(3)	4+(3)	4+(3)	±(1) 2+(2) —(8)	4+(6)	4+(6)	4+(10)	4+(3)	4+(7)	(a)
I -----	4+(2)	4+(2)	4+(3)	4+(4)	—	4+(4)	—	4+(4)	2+(10)	4+(7)

NOTE.—Reagents unfermented in the 0.5 per cent series: Arabinose, galactose, salicin, saccharose, amygdalin, dextrin, sorbite, inosite, lactose, raffinose, adonite, dulcite, and nutrose. Arabinose and galactose were fermented in the 1 per cent series.

<sup>a</sup> Negative in two tests with this lot of medium.

In the 0.5 per cent agars dextrose and mannite were as promptly and as completely acidified as in the serum waters. Levulose, however, showed a constant and complete acidification, and maltose was much more markedly changed, though with the three New Orleans human strains there was no reaction. With two of the strains obtained locally (*F* and *H*), there was a partial acidification with subsequent reversion; in both instances the reddening occurred only after a heavy, primarily nonacid-producing growth had developed, and then was confined to the

medium under small parts of the growth. That this localized acid production was temporary is indicated by the fact that when sufficient time had elapsed for the reaction of the medium to become uniform throughout by diffusion the entire slant again became blue.

With 1 per cent agars the strains inactive in series I showed fermentation. Arabinose cultures also showed more marked acidity, but the galactose reactions were still irregular and not satisfactory. With salicin there was complete inactivity with three strains, while the glycerin cultures, instead of reacting more rapidly, were somewhat delayed in attaining complete change. These results emphasize the fact that litmus-sugar agars, even of 1 per cent concentration, are not satisfactory for the demonstration of acid production by organisms such as *B. pestis*, the fermentation activities of which are of low degree.

#### SUGAR BOUILLONS, PHENOLPHTHALEIN TITRATIONS

In order to establish quantitatively the fermentative power of the different strains, and also to determine the relation between actual acid production by *B. pestis* and the indication of such activity given by the media previously employed, a complete series of broth cultures was made in bouillons containing the eighteen carbohydrates previously used.

Each tube contained from 15 to 20 cubic centimeters of 0.5 per cent sugar bouillon, made with beef extract (Liebig's) in the ordinary manner. This was used because the pest organism grows fairly well in it and because preliminary tests had shown that no change in reaction detectable by titration occurs from growth of *B. pestis* in broth so made. The tubes of the first series contained small, inverted "fermentation tubes." After three days, no gas having appeared, these were removed. After ten days of total incubation they were heated in an Arnold sterilizer for twenty minutes before being titrated. The estimations were made, using N/20 and N/50 sodium hydroxide, respectively, in the two series tested. Hot titration was used by choice, in spite of the fact that it gives somewhat higher readings than are obtained in the cold. Accuracy of comparison was considered of first importance rather than the exact determination of absolute acid increase.

Two series were titrated, as upon titration the first lot of media was found to have been between 1.5 and 2.2 per cent acid. The results obtained in series I agree in the main with those of the second series, which was adjusted to about 0.3 per cent acid before sterilization.



In Table III are shown the amounts of acid increase by each strain in those sugar bouillons of series II that were in any degree affected. There is also shown the average per cent of acid production in each sugar, with the similar averages of series I appended for comparison. Further, as indicating the relative fermentative activities of the different strains, the average acid production by each strain of organism in all but the glycerin medium is shown.

TABLE III.—Increase of acidity in 0.5 per cent sugar bouillons (series II) in cubic centimeters of normal sodium hydroxide.

Strain.	Dex-trose.	Man-nite.	Levu-lose.	Malt-ose.	Salicin.	Arabi-nose.	Galac-tose.
A -----	1.5	1.7	1.3	1.2	1.8	1.2	1.2
B -----	1.5	1.7	1.4	0.8	1.8	1.0	0.9
C -----	1.6	1.5	1.2	1.1	1.8	1.0	0.8
D -----	1.5	1.6	1.6	0.6	1.9	1.0	0.8
E -----	1.4	1.7	0.8	1.1	1.6	1.1	0.8
F -----	1.4	1.4	1.3	1.1	1.6	0.8	0.9
G -----	1.5	1.3	1.1	1.0	0.4	1.2	0.3
H -----	1.4	1.5	1.1	1.0	1.1	1.2	0.7
I -----	1.7	1.7	(a)	1.1	1.8	1.3	0.9
Average increase -----	1.50	1.57	1.22	1.00	1.53	1.09	0.80
Average increase, series I -----	1.22	1.02	1.64	0.82	0.81	0.79	0.52

Strain.	Dex-trin.	Inosite.	Sorbite.	Saccha-rose.	Amyg-dalin.	Glyc-erin (1 per cent).	Aver-age ac-tivity.
A -----	0.5	0.1	0.1	0	0	0.6	0.82
B -----	0.4	0.1	0.2	0	0	0.1	0.75
C -----	0.5	0	0.1	0	0	0	0.74
D -----	0.5	0	0.1	0.1	0	0.2	0.75
E -----	0.5	0	0.2	0	0	0.1	0.71
F -----	0.5	0	0.1	0.2	0.1	0.1	0.72
G -----	0.4	0	0	(b)	0	1.1	0.60
H -----	0.4	0	0.1	0	0	0.4	0.67
I -----	0.5	0	0.3	0.3	0.3	0.2	0.84
Average increase -----	0.47	0	0	0	0	(c)	0.73
Average increase, series I -----	0.35	0.25	0.31	0.41	0.37	-----	-----

<sup>a</sup> No growth.

<sup>b</sup> Contaminated.

<sup>c</sup> Irregular.

As a result of these titrations the reagents used may be roughly grouped according to the extent to which *B. pestis* produced acid in bouillons containing them. Group I includes dextrose, mannite, and levulose, which are strongly acidified. In group II are salicin, arabinose, maltose, and galactose, which are weakly acidified. Glycerin holds an odd position in that but two of the

strains here used (*G* and *H*) ferment it. To these should be added strain *J*, which was obtained after this phase of the work was completed, but which shows acid production in other glycerin media. The reaction with dextrin may be due to traces of dextrose; at any rate, being less than 0.5 per cent, it is so slight as to seem negligible.

With regard to the second group it is interesting to note that in spite of the constantly negative results in the litmus media with all of these but maltose, and the negative serum water and irregular agar reactions even with this sugar, their bouillons show a constant, regular acidification ranging from 0.6 to 1.9 per cent.

While there is no distinction between American and Philippine strains, there will be noted in the averages slight variations in the total acid production by the different strains. Thus the old New Orleans rat strain (*A*) and one of the Bureau of Science strains (*I*) cause considerably higher acidification than the others, while one of the Philippine strains in particular (strain *G*) shows a constantly comparatively low acid tolerance.

#### INFLUENCE OF SUGAR AND MEDIUM ON MORPHOLOGY

The morphology of *B. pestis* has been so often and so exhaustively described that but a few points in connection with the influence of the different sugars used merit brief notice.

*Three-day sugar-agar cultures.*—All smears examined were stained with Loeffler's blue. On dextrose, mannite, and levulose, in which media fermentation begins promptly, smears uniformly show much degeneration and involution. When acid production appears late, the degeneration and involution occur to a less degree. The cultures on sugars not acidified give well-stained organisms, though variation in morphology in different instances is marked. The same sugar, however, seems in the majority of instances to produce somewhat similar effects in the different strains. Salicin, for instance, usually produces long, often thready and at times almost filamentous organisms (*B. proteus* type), not at all recognizable as *B. pestis*. Dextrin and arabinose also show this tendency. On the other hand, glycerin induces fairly constantly the formation of short, chunky, deeply staining bacilli, often showing the typical bipolar appearance of *B. pestis* in exudates. In a very few instances there were encountered long pale organisms with deeply stained polar bodies, indistinguishable from *B. diphtheriæ* under the blue stain.

*Ten-day sugar-bouillon cultures.*—The morphology in dextrose and mannite bouillons differs as widely from that on the similar

agars as do the quantities of growth on these media. Here is found with regularity extensive, typical chain formation, often with an almost sheathlike capsular layer. In media containing substances such as glycerin and arabinose, which are not fermented and in which growth is not heavy, chain formation is not a feature and loose bacillary forms predominate. Stalactites do not develop in such media. Galactose bouillon, it may be noted, produces typical, short, bipolar forms rather more constantly than any other. Filamentous forms occasionally appear which, particularly when tangled, resemble streptothricial organisms; they do not occur regularly enough to seem characteristic features of any particular medium.

#### INFLUENCE OF SUGAR AND MEDIUM ON AMOUNT OF GROWTH

The amount of growth in these sugar media varies widely, and the effect of the same sugar differs remarkably, depending on the type of medium. In the bouillons the growth is usually heaviest in those in which acid production is most marked and the lighter growths occur with those sugars not affected. Thus in dextrose bouillon, for instance, the growth is very heavy, with a coarse, flocculent deposit on the bottom and the sides. On dextrose agar, however, the reverse holds true, the growth here being very quickly inhibited by the acid produced. In the bouillons dextrose, mannite, and levulose have given the heaviest development; maltose, dextrin, and galactose somewhat less; while only fair amounts of growth occurred in the remaining sugars except amygdalin, sorbite, and glycerin, which seemed distinctly unfavorable. These differences were more marked after three than after ten days.

With the agar media, growth seems promptly to cease once the underlying medium becomes acidified. If this occurs early, the growth is very light; if later, it is correspondingly heavier. Whenever reversion to neutral occurs, the growth, having been temporarily retarded, goes on to maximum. Due to the operation of this rule the growths of the series I maltose set, in particular, varied widely.

In none of the serum waters has the growth appeared heavy, though from the nature of the material it is difficult to observe this feature with accuracy.

#### ANALYSIS OF FERMENTATION REACTIONS

Consideration of the inconstant and conflicting reactions obtained by the use of certain of these media brings up the question of the several factors concerned in the irregularities. These

are of more or less interest and importance, for while with some organisms fermentation is so active and definite that results are clear-cut under usual conditions, others, among which *B. pestis* is to be included, are less active in this respect and are, therefore, prone to irregularities of result unless the conditions of experiment are properly controlled.

Different types of media necessarily present different conditions, such as essential suitability, aëration, availability of contained reagent, and diffusion of end product, all of which may affect the metabolism of the organism under consideration. Beside these, differences in indicators must also be considered. In order to demonstrate the influences of certain of these various factors, a considerable number of tests have been carried out.

#### THE INFLUENCE OF TYPE MEDIUM ON FERMENTATION

The tests described above demonstrate that for *B. pestis*, which in most instances is a weak fermenter, the nature of the medium used plays an important rôle. Comparison of the numbers of sugars fermented in each type emphasizes the superiority of bouillon, as has repeatedly been shown for other organisms. Litmus-agar slants under certain conditions are almost as useful, but Hiss's serum water is of much less value, and the results with it are more misleading. These comparisons are summarized in Table IV, which is compiled from the results already shown together with certain reactions yet to be discussed.

TABLE IV.—Summary of carbohydrate fermentation by *B. pestis* in various media, with 0.5 and 1 per cent concentration of sugars.

[Symbols indicating degree of acid production: ++=strong and constant; +=definite and usual; ±=weak and irregular but usual; ∓=unusual and weak; —=none.]

Medium.	Litmus serum-waters.		Litmus agars.		Bouillon (titrations).		Remarks.
	0.5 per cent sugars.	1.0 per cent sugars.	0.5 per cent sugars.	1.0 per cent sugars.	0.5 per cent sugars.	1.0 per cent sugars.	
Dextrose .....	++	—	++	—	++	—	Strong.
Mannite .....	++	—	++	—	++	—	Do.
Levulose .....	±	+	±	—	++	—	Do.
Maltose .....	∓	+	±	+	++	++	Moderate.
Arabinose .....	(∓ ?)	+	—	+	+	+	Weak.
Galactose .....	—	∓	—	±	+	+	Do.
Salicin .....	—	±	—	+	+	+	Do.
Glycerin .....	—	—	∓	∓	∓	∓	Irregular.
Dextrin .....	—	—	—	—	(± ?)	(± ?)	Negative.

Tests with the following were clearly negative: Lactose, adonite, inosite, saccharose, dulcité, sorbite, raffinose, amygdalin, nutrose, and inulin.



From this summary may be drawn definite conclusions as to the fermentation activities of *B. pestis* in the media used.

#### LIMITATION OF ACID TOLERANCE

In the 0.5 per cent bouillon series (Table III) the cultures, irrespective of the nature of the sugar, the extent of growth, or the primary acidity, showed a maximal acidity of about 2.5 per cent, which reaction was obtained with several sugars. This indicates a rather remarkably constant limit of acid tolerance for the various strains.

*The influence of concentration of carbohydrate on end reaction.*—That increase of sugar, up to a certain point at least and under certain conditions, causes an increase in the rapidity or the degree of the reaction is axiomatic. The effect of variation in amount depends, to a certain extent at least, upon the activity of the organism against the particular sugars used. For instance, 0.5 per cent dextrose serum-water was as completely acidified as was the 1 per cent medium. On the other hand, 1.5 or 2 per cent maltose or arabinose serum-waters would probably have reacted more strongly than did those of 1 per cent concentration to be shown in Table VI. In order to demonstrate whether widely different concentrations of sugar would have any influence on the final acidity, a series of peptone-water cultures was made, dextrose and mannite being the sugars used. Three strains of organisms with somewhat different fermentation activities were selected. The titration results appear in Table V.

TABLE V.—*Final acidity produced in peptone waters containing different amounts of carbohydrates.*

	Strain—			Control (average of two).
	B.	G.	I.	
Dextrose:				
0.5 per cent .....	2.00	1.72	1.92	0.68
1.0 per cent .....	2.16	1.80	1.88	0.76
1.5 per cent .....	2.16	1.92	2.04	0.84
2.0 per cent .....	2.24	1.84	1.92	0.88
2.5 per cent .....	2.16	2.00	2.12	1.00
Mannite:				
0.5 per cent .....	1.76	1.82	1.44	0.54
1.0 per cent .....	1.68	1.82	1.48	0.50
1.5 per cent .....	1.8	1.82	1.44	0.52
2.0 per cent .....	1.75	1.40	1.00	0.44
2.5 per cent .....	1.84	1.82	1.48	0.48

These results show that a considerable variation in concentration of sugars has no appreciable effect on the total acid produced and that here, as elsewhere, the acid production by any one strain with the same sugar is remarkably constant. There is again demonstrated, however, a consistent although slight difference among the strains.

In order to obtain direct evidence as to whether the acidities attained in the 0.5 series had in any degree been determined by the low sugar concentration, a number of 1 per cent sugars was similarly tested. To learn, further, whether a simpler nutrient vehicle for the carbohydrates would modify the amount of acid produced, a small series was also tested in a solution containing 2 per cent peptone and 0.5 per cent sodium chloride. The results of these series are included in Table VI.

TABLE VI.—Total acidities attained in 1 per cent carbohydrate fluid media, in terms of cubic centimeters of normal sodium hydroxide.

Strain.	Bouillon 1 per cent. <sup>a</sup>					Peptone waters, 1 per cent. <sup>a</sup>			
	Levu-lose.	Malt-ose.	Salicin.	Arabin-ose.	Galac-tose.	Malt-ose.	Salicin. <sup>c</sup>	Arabin-ose.	Galac-tose.
A -----	2.15	1.7	1.7	2.0	1.85	2.3	0.75 (0.05)	2.5	2.4
B -----	2.3	1.5	1.8	2.0	1.9	2.5	1.15 (0.45)	2.65	2.4
C -----	2.2	1.7	2.0	2.2	1.8	0.95	0.75 (0.05)	2.75	2.2
D -----	2.2	1.55	1.7	2.05	1.85	1.5	0.75 (0.05)	2.65	2.8
E -----	2.1	1.6	1.25	2.1	1.8	2.5	0.95 (0.25)	2.45	2.85
F -----	(b)	1.65	2.3	1.95	1.65	1.9	0.9 (0.20)	2.0	2.3
G -----	1.8	1.45	1.2	1.7	1.3	1.35	0.85 (0.15)	1.95	1.55
H -----	2.1	1.45	1.0	2.05	1.7	2.35	0.7 (0.0)	1.85	1.45
I -----	2.1	1.65	(b)	2.1	1.75	2.35	0.85 (0.15)	2.2	2.1
J -----	2.0	1.2	1.15	1.35	1.85	1.3	0.9 (0.20)	1.95	2.5
Controls:									
1 -----	1.00	1.00	0.55	1.25	0.80	1.00	0.65	1.80	1.10
2 -----	1.00	1.00	0.45	1.15	0.90	0.90	0.70	-----	1.15

<sup>a</sup> Titrated with test N/50 sodium hydroxide.

<sup>b</sup> No growth.

<sup>c</sup> Figures in parentheses represent apparent increase in acid.

Comparison of these results with those given in Table III shows that in no case was there a radical increase of acidity in the 1 per cent series. Contrasting the bouillon and peptone

cultures, it seems that fermentation in the peptone media was usually rather more active than in the corresponding bouillon. Of interest, however, is the fact that salicin fermentation in peptone water is remarkably depressed, this being similar to the results obtained in serum water. The uniformity in the amount of acid formed in the same medium by the different strains is again well shown. There is sometimes, however, a distinct difference in the end reaction in the different sugars, an indication, possibly, of different end products which are inhibitory in different concentrations.

*Influence of primary acidity on end reaction.*—In connection with inhibition of fermentation the question arises whether a simple primary acidity, due to a known substance, might not be tolerated to a higher degree than the mixed products of bacterial metabolism. To investigate this point, two series of cultures of different reactions were made by adding hydrochloric and lactic acids to neutral broth. Dextrose and mannite were again used for the carbohydrates. The results of these titrations indicated that fermentation is inhibited as quickly by hydrochloric or lactic acid as by the products of the organism's own metabolism. No difference whatever was demonstrated in the tolerance to the two acids.

*Acid limit with meat-infusion broths.*—In the foregoing titrations the maximal acidity attained was between 2.5 and 3 per cent to phenolphthalein. This is not the highest total acidity tolerable to *B. pestis*, for cultures in sugar broths made up of sugar-free veal infusion attain a considerably higher acidity than has been the case in beef-extract broths under any circumstance.

TABLE VII.—*Final reactions attained in 1 per cent veal-infusion sugar broths.*

Strain.	Dextrose.	Mannite.	Maltose.
A .....	4.0	4.2	3.5
B .....	4.1	4.0	4.2
C .....	4.2	4.2	2.2
D .....	4.2	4.1	4.0
E .....	4.2	3.8	3.7
F .....	3.8	3.8	4.0
G .....	4.2	4.1	3.5
H .....	3.8	4.1	3.8
I .....	4.0	3.5	3.7
J .....	4.0	4.3	2.1
Control titrations:			
1 .....	1.6	1.3	1.7
2 .....	1.3	1.3	1.7

The figures in Table VII demonstrate that *B. pestis* in the veal-infusion broths tolerates acid up to about 4 per cent (3.8 to 4.2), which is 1 to 1.5 per cent higher than in beef-extract media.

THE INFLUENCE OF TEMPERATURE AND OXYGEN PRESSURE ON RATE OF FERMENTATION

It is a recognized fact that the temperature at which *B. pestis* is cultivated influences the organism to a considerable degree. In consideration of this the rates of acid production at incubator and room temperatures were compared. In Table VIII are shown the end reactions in 1 per cent Hiss's serum waters containing levulose, maltose, salicin, arabinose, galactose, and glycerin. The room temperature ranged from 27 to 30° C. during the period of cultivation.

TABLE VIII.—Comparison of fermentation of 1 per cent sugar serum-waters at incubator and room temperatures.

[End result after ten days; symbols as in Table I; the day of observation in parentheses.]

Strain.	Levulose.		Maltose.		Salicin.		Arabinose.		Galactose.		Glycerin.	
	37°.	28°.	37°.	28°.	37°.	28°.	37°.	28°.	37°.	28°.	37°.	28°.
A .....	4+ (4)	4+ (3)	4+ (5)	4+ (3)	+ (10)	+ (4)	4+ (4)	4+ (3)	2+ (3)	4+ (4)	—	—
B .....	4+ (3)	4+ (3)	3+ (9)	4+ (4)	± (4)	+ (6)	2+ (5)	4+ (4)	+ (2)	4+ (10)	—	—
C .....	4+ (3)	4+ (3)	1+ (4)	4+ (3)	—	3+ (9)	2+ (9)	4+ (9)	+ (2)	2+ (9)	—	—
D .....	4+ (3)	4+ (3)	4+ (3)	4+ (2)	± (4)	3+ (9)	4+ (4)	4+ (3)	+ (2)	4+ (5)	—	—
E .....	3+ (7)	4+ (3)	1+ (4)	4+ (3)	—	+ (6)	+ (4)	4+ (5)	+ (3)	2+ (5)	—	—
F .....	4+ (4)	4+ (3)	1+ (4)	4+ (2)	1 (9)	4 (7)	+ (5)	4+ (5)	+ (2)	+ (3)	—	—
G .....	+ (3)	4+ (4)	1+ (8)	4+ (4)	—	—	2+ (9)	4+ (6)	+ (5)	2+ (9)	—	+ (8)
H .....	4+ (3)	4+ (3)	4+ (3)	4+ (3)	—	± (4)	4+ (4)	4+ (4)	+ (3)	+ (3)	+ (4)	+ (5)
I .....	2+ (5)	4+ (3)	1+ (4)	4+ (2)	± (10)	+ (8)	+ (5)	4+ (5)	+ (2)	+ (3)	—	—
J .....	+ (8)	4+ (3)	4+ (5)	4+ (3)	—	—	—	± (3)	+ (3)	4+ (4)	—	—

The serum-water cultures represented in Table VIII evidenced a considerably greater activity at the room temperature, at which saprophytism is pronounced, than at 37° C., the temperature of human-body parasitism.

Similar, though somewhat less marked, is the difference in effect of these temperatures with litmus agars. In practically every instance acidification began more quickly on the room-temperature slants and in most instances became complete sooner. Further, in several cases it became complete only at room temperature. With 0.5 per cent agars several maltose cultures in the incubator became partially acid and reverted to neutral, while the corresponding room-temperature cultures became and remained strongly acid.



Two sets of agar slants, maltose and glycerin, were cultivated in vacuo at body temperature in a Novy jar. The reactions were at first accelerated to equal those of the room-temperature control cultures, but at the end of a week the cultures showed little difference from the aërobic incubator controls. Later duplicate sets of 1 per cent maltose serum-water and litmus agar were similarly cultivated. Here there was definitely, although but slightly, more active fermentation in the anaërobic sets, the advantage persisting through the four days for which the cultures were observed.

FERMENTATION IN HISS'S LITMUS SERUM-WATER

*Effect on medium of directly added acids.*—Comparison of the reactions of *B. pestis* in serum-water cultures and on litmus agars is distinctly unfavorable to the former, though it would seem that, so far as the indicator is concerned, the results should coincide. The possibility that a so-called "buffer" absorption of acid by the serum itself might play some part in the delayed reaction was considered. To determine this, increasing amounts of dilute hydrochloric and lactic acids were slowly added to measured volumes of sugar serum-waters, the tubes being agitated the while. The results were practically identical in several tests. The amounts of acids required to produce the changes are small and regular, and the "buffer" effect is, at the most, but 0.1 to 0.2 per cent. The acidity required to produce various stages represented heretofore by the "plus system" is shown in Table IX. By the application of these equivalents, the readings in Tables I and VIII might be made roughly quantitative.

TABLE IX.—Changes produced by adding dilute acids to Hiss's litmus serum-water, as determined with hydrochloric and lactic acids.

Normal acid.	Appearance with transmitted light.	Appearance with reflected light.	Equivalent to symbols as used.
<i>Per cent.</i>			
0.1	None, or faintest perceptible change ..	No change.....	—
0.2	Slight reddening .....	No change.....	±
0.3	Red predominant.....	Faint reddening.....	
0.4	Red almost pure .....	Reddening more distinct .....	+
0.5	(About as above) faintest clouding ..	(About as above) .....	
0.6	Red practically pure; opacity developing.	Red and blue about equal; precipitation not detectable.	2+
0.7	Red and opaque, but fluid.....	Red practically pure; opacity evident.	3+
0.8		Coagulated.....	4+
0.9			
1.0	Coagulated .....		

*Acid production in serum bouillon.*—Another question to be determined was whether unsatisfactory results with serum waters were due to limitation of growth on account of the simplicity of the medium, or to the possibility that the serum itself actually exercises a restraining effect. If the latter were the case, sugar peptone-water with varying concentrations of added serum should show differences in fermentation. A quantity of dextrose peptone-water was separated into five lots, to four of which Berkefeld-sterilized horse serum was added in various concentrations. These and a control set without serum were inoculated, using all ten strains of *B. pestis*. Titrations were made with N/40 sodium hydroxide, after six days' incubation in this case. The results appear in Table X.

TABLE X.—*Acid production in dextrose peptone-water containing various amounts of filter-sterilized horse serum.*

Strain.	Serum.				Control without serum.
	0.2%.	1.0%.	5.0%.	10.0%.	
A .....	2.65	2.5	2.75	2.5	2.6
B .....	2.6	2.6	2.55	2.6	2.3
C .....	2.6	2.55	2.75	2.8	2.65
D .....	2.5	2.55	2.65	2.65	2.55
E .....	2.2	2.4	2.65	2.35	2.25
F .....	2.4	2.65	2.7	2.5	2.25
G .....	2.5	2.75	2.6	2.4	2.5
H .....	2.65	2.75	2.75	2.8	2.25
I .....	2.2	2.2	2.5	1.8	2.0
J .....	2.35	2.4	2.65	2.5	2.15
Uninoculated controls .....	0.6	0.6	1.05	1.3	0.65
	0.75	0.8	-----	0.95	0.19

No depression of fermentation is demonstrated in these cultures, indicating that serum per se, in these percentages at least, is not inhibitory, despite the fact that the amount of growth was somewhat less than in dextrose peptone-water without serum.

*Influence of added nutrient on the reaction with Hiss's serum waters.*—Since serum per se appears not to have an inhibitory effect, the effect of adding simple nutrient substances to the serum-water stock was investigated. Beef extract alone proved to be of no value. Peptone, if added in 1 per cent concentration to the previously heated serum, gives, upon reheating, a soft jelly, as described by Buerger,<sup>(19)</sup> which physically is not the same as Hiss's medium. Making the original serum water one part of serum to four of water and adding 0.5 per cent of peptone after the primary heating gives an enriched medium which is

similar to Hiss's in appearance and reaction. Its value, however, seems but moderately greater than the ordinary serum water. With some sugars, as levulose and maltose, the reaction in the peptonized cultures was considerably accelerated, but with galactose the two media gave identical results, and with salicin no fermentation occurred in either.

#### SUMMARY AND DISCUSSION

Cultivations carried out with ten strains of *B. pestis* in media containing twenty fermentable substances have demonstrated conclusively the fermentation activities of the organism. Experiments have also demonstrated the parts played by certain influencing factors in the results obtained. As shown in Table IV, dextrose, mannite, and levulose are regularly and strongly fermented, while maltose, arabinose, galactose, and salicin are less constantly acted upon except in the more suitable media. Glycerin, which Vourland and Schöbl found to be fermented, in my experience reacted with but three strains out of ten, and then only under suitable conditions. This difference between the strains seems to have no significance. Contrary to the conclusion of MacConkey, dextrin is not fermented; contrary, similarly, to Schöbl and to Vourland, saccharose remains untouched. Salicin fermentation is peculiar in that it is practically negligible in serum water and in simple peptone media, although positive in agar and bouillons.

The strains used included a culture isolated in 1912 from a rat in New Orleans, three from human cases in the 1914 New Orleans invasion (suggested at the time by the Federal authorities to have come from Liverpool), five isolated from cases in the Philippine Islands, and one avirulent strain. Between these there has been demonstrated no distinct difference except for the irregularity with glycerin noted and except that between the strains there are consistent although slight differences in acid tolerance. This is shown numerically in Table III, where the relative "activities" of the strains in bouillons have been averaged. Strains *G* and *H* produce somewhat less, and strains *A* and *I* slightly more, acid than the majority. These variations are evidently not dependent upon length of time out of the animal body. The relation to virulence has not been investigated, but it is probably true that, as recently asserted by Berlin, this feature, also, has no definite influence.

There has been demonstrated a fairly constant point of maximum acid tolerance beyond which active metabolism as expressed in fermentation ceases. This is a well-recognized feature



of bacterial fermentation. *Bacillus coli*, for instance, as shown by Browne,<sup>(20)</sup> has a similarly uniform maximal point, which lies between 2.1 and 2.4 per cent in dextrose and somewhat lower with some sugars and is not raised by increase in sugar present, or in the total amount of medium, or by increase in initial acidity. Streptococci are quite different in that the maximal point of acid toleration differs greatly in different strains. Broadhurst's figures,<sup>(21)</sup> obtained from sugar-free, meat-infusion cultures, ranged between 1.5 and 8.5 per cent. In a beef-extract broth series<sup>(22)</sup> the highest that she obtained was 2.3 per cent as compared with 5.0 and 5.2 per cent with parallel infusion broths, though with meat-extract broths Fuller and Armstrong<sup>(23)</sup> obtained much higher figures. Hopkins and Lang<sup>(24)</sup> concluded that—

Fermentation by a given streptococcus ceases when a certain acidity is reached, irrespective of how much acid must be formed to produce this acidity.

So far as can be learned, the maximum acid production by *B. pestis* has not previously been noted, although the limit of primary acidity of media permitting the growth of the organism has been studied. Wladimiroff and Kressling<sup>(25)</sup> found that the addition of small amounts of acid diminished the amount of growth, 3 per cent of normal hydrochloric acid causing complete inhibition. Lactic acid was better tolerated, 5 per cent being the maximum. Pakes and Joseph<sup>(26)</sup> found the point of inhibition of growth to be as high as 4.0 to 4.5 per cent. My results, using sugar bouillons of different degrees of acidity, do not coincide with this, as there was little growth and no increase in end reaction with media primarily 2.5 per cent acid or above, hydrochloric and lactic acids having identical limits.

It is shown that the maximum acidity produced by *B. pestis* in beef-extract bouillons ranges between 2.5 and 3 per cent. This seems to be the same for all strains, although (see particularly Table V) a strain may have a slightly lower maximum point with one sugar than with another, due possibly to the special nature of the end products. In some cases (see Table VI) all strains uniformly show this feature, which has often been noted in carbohydrate-fermentation studies.

Similarly to Broadhurst's results with streptococci, veal-infusion broths permit, for some reason not determined, a greater accumulation of titrable acid before fermentation is inhibited. This seems probably due to differences in the nature of secondary end products. The figures obtained in the series detailed in



Table VII show the maximal point under these conditions to be between 3.5 and 4.1.

Beyond the point of sugar concentration required under the conditions obtaining to bring the total acid produced up to the point of tolerance, addition of more sugar does not increase the final acidity. In several instances (see Table IV) 0.5 per cent of reagent has proved insufficient to secure maximum fermentation. Further, in a number of instances (as in Table II) reversion to neutral from incomplete acidification of 0.5 per cent agar slants occurred.

Comparative cultivations show that room temperature, which was used by Schöbl in his tests, influences fermentation to considerably greater activity than does body temperature. Anaërobic cultivation at the latter heat accelerates, at least temporarily, acid formation sometimes even more than does aërobic cultivation at room temperature.

The morphology of the organism varies more or less with the sugars, depending not only upon the involuting and degenerating influence of the acid sometimes formed, but also upon other less evident influences where acid is not produced. The extent of growth is also considerably modified, apparently anomalously in different media. In bouillons containing fermentable substances the growth is most luxuriant; on agars containing the same reagents the growths are much less heavy than on unacidified media. In bouillon, because of its fluidity, acid when formed cannot inhibit growth until such time as the entire mass of the medium is brought to the maximum point of acid tolerance. In agar, on the other hand, as soon as the medium directly beneath the growth becomes sufficiently acidified, multiplication seems almost to cease. Further production of acid constantly replaces that lost by diffusion to the deeper levels, and the growth remains light. Thus under different physical conditions the same carbohydrate produces opposite effects. Occasionally, when acid production in the agar culture is weak, reversion occurs and the primary, light, acid-producing growth is replaced by a heavy, nonfermenting growth.

Of the three methods of determining acid production, titration of sugar bouillons is by far the best, as is shown particularly by the contrast between the negative or weak and irregular fermentation of salicin, arabinose, galactose, and maltose in the other media and their regular fermentation in bouillons. Only in bouillons did the 0.5 per cent series approximate the 1 per cent series in regularity or intensity of reaction. In sugar

peptone-waters a slightly greater amount of acid sometimes developed than in the corresponding bouillons.

The degrees of acidity required to show change in litmus serum-waters may be demonstrated by adding very dilute normal acids to measured quantities of the medium. Acid up to or slightly above the equivalent of 0.1 per cent usually causes no reaction, an inertia due possibly to the "buffer" effect of serum, which Levy and Rowntree<sup>(27)</sup> found to be from 0.1 to sometimes 0.3 per cent for fresh serum, which amount of acid could be added without raising the hydrogen ion concentration. This margin is too slight to be responsible for the occasional apparent fermentation inactivities in serum media. Faint change usually occurs with between 0.1 and 0.2 per cent acidification, while coagulation is complete with less than 1 per cent, the reactions being identical with hydrochloric and lactic acids. The different changes are brought about regularly in different lots of the medium by fairly definite amounts of acid, which makes the observation of such reactions of approximate quantitative value up to the point of coagulation of the serum.

The unmodified litmus serum-waters of Hiss, although valuable in the identification of intestinal and of other organisms, have repeatedly proved unreliable and misleading with *B. pestis*. Similar experiences have been had by other observers with bacteria of low fermentation activity, as for instance certain streptococci. That the reactions obtained do not result from a directly inhibitory effect of the serum is indicated by the fact that no suggestion of inhibition was shown in bouillons to which serum had been added. Further, the addition of peptone sometimes increases the reaction, although this acceleration is not constant enough with the different sugars to make such a medium of general value in a study of bacterial fermentation.

#### CONCLUSIONS

Comparison has failed to demonstrate any distinct difference, qualitative or quantitative, between the fermentation activities of Oriental (Philippine) and certain American strains of *B. pestis*. There is, on the other hand, a rather remarkable agreement between the different strains except solely with regard to glycerin fermentation.

Under usual conditions dextrose, mannite, and levulose are fermented regularly and fairly strongly. Maltose, arabinose, galactose, and salicin are also fermented, but more irregularly except under favorable conditions. A few strains ferment glycerin. Dextrin, lactose, saccharose, raffinose, adonite,

dulcitate, amygdalin, inositol, sorbitol, nutritose, and inulin are not changed. The division of the group into glycerin fermenters and nonglycerin fermenters has no apparent significance.

Veal-infusion bouillon is the most suitable medium for carbohydrate fermentation by *B. pestis*. Litmus agar is more favorable than Hiss's litmus serum-water unmodified, which is quite unsuitable. Diminished reaction in Hiss's serum-waters is due to unsuitability of the medium for luxuriant growth of the organism and not to any directly inhibiting effect of serum per se. In it, whether plain or peptonized, such reagents as appear to be not adapted to promote growth by being themselves primarily utilized are fermented secondarily and less decisively. Salicin seem peculiarly unfermentable by *B. pestis* in serum water or peptone water in usual concentrations.

The types of media and the different sugars used have various effects, some of them very definite and constant, on the amount of growth and on the morphology of the organism.

Fermentation ordinarily occurs more rapidly and completely at room temperature (27° to 30° C.) than at body temperature. Under anaërobic conditions at 37° C. acid production is accelerated, temporarily at least, to exceed the aërobic, room-temperature reactions.

There is a well-defined maximal point of acid tolerance, which is fairly uniform in the same medium for the different strains. This ranges from 2.5 to 3 per cent acid for beef-extract broths and 3.8 to 4.2 per cent with veal-infusion broths. There are slight differences in the highest acidities with the different carbohydrates. The maximal point is not changed by increased percentage of the sugar or differences in the original reaction.

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## REVIEWS

The Mortality | from Cancer throughout | the World | by | Frederick L.  
Hoffman, LL. D., | F. S. S., F. A. S. A. | [5 lines] | Newark, New  
Jersey | The Prudential Press | 1915 | Cloth, pp. i-xv + 1-826.

The following is an excerpt from the preface by the author:

"The work is divided into nine chapters, to all but one of which there is an appendix of forms or tables, which, as a matter of convenience, have been placed together at the end of the volume. Chapter I, on The Statistical Method in Medicine, is amplified by an appendix of the principal cancer classifications, past and present, used in standard textbooks and in the compilation of international cancer mortality statistics. \* \* \* Chapter II, on The Statistical Basis of Cancer Research, is a brief discussion of the fundamental statistical facts available for analysis, enlarged by an appendix of the blanks and certificates used in connection with cancer mortality investigations and special research, including the question form for cancer census purposes recommended by the International Association and the special blanks for supplementary inquiries into the facts and circumstances connected with the occurrence of cancer of the uterus, mammary cancer, gastric cancer and cancer of the buccal cavity, adopted and recommended by the Statistical Committee of the American Society for the Control of Cancer, in coöperation with the General Memorial Hospital of the City of New York. Chapter III, on The Increase in Cancer, is an extended discussion of the general problem of the observed upward tendency of the cancer death rate throughout the world. \* \* \* The Mortality from Cancer in Different Occupations is discussed in Chapter IV, with an appendix of eight tables of the mortality from cancer in selected industries and employments, derived from the decennial reports of the Registrar-General of England and Wales, but rearranged and recalculated for the present purpose. In addition, the appendix includes cancer mortality data by occupations, derived from the industrial mortality experience of The Prudential and the cancer census of Hungary. Chapter V presents an extended discussion of Cancer as a Problem in Life Insurance Medicine, historically and practically considered, with an appendix of 121 tables, including a concise and uniform presentation of the general cancer experience data of a large number of American and foreign life insurance companies and the collective results of the Medico-Actuarial Mortality Investigation.

Chapter VI, on the Geographical Incidence of Cancer Throughout the World, brings out forcibly the wide range in the cancer frequency rates of different countries and cities with widely varying circumstances of race, climate, habits, etc., all of which are shown to have an important bearing upon the cancer problem as a whole. \* \* \* In Chapter VII, on The Statistical Data of Cancer Frequency in American States and Cities, the rate of cancer occurrence throughout the United States is discussed at some length, and amplified by an appendix of 259 tables of cancer mortality for the registration area and for the several states and cities in a uniform manner and with a due regard, as far as practicable, to the elements of age, sex, race, organs and parts, etc. Chapter VIII presents the corresponding information on The Statistical Data of Cancer Frequency in Foreign Countries, with an appendix of 389 tables for countries other than the United States. Chapter IX concludes the results of the statistical inquiry with Some General Observations and Conclusions on the Cancer Problem. This is a general discussion of practically all the more or less controversial aspects of the cancer question, with a first regard, however, to sociological, anthropological and general scientific consideration. \* \* \* The appendix to this chapter includes reprints of suggestive educational circulars used in connection with the nation-wide propaganda for cancer control under the auspices of the American Society for the Control of Cancer, etc."

A bibliography of the important works and articles on cancer is included.

Differential diagnosis | volume II | [2 lines] | by | Richard C. Cabot, M. D.  
[3 lines | ornament | 1 line] | profusely illustrated | Philadelphia  
and London | W. B. Saunders Company | 1915 | Cloth, pp. 1-709.  
Price, \$5.50; half morocco, \$7.00.

Cabot's Differential Diagnosis, Vol. ii, has filled a long-felt want in differential diagnosis. Exhaustive analyses on the frequency of certain symptoms, with diagnosis, have been made by the author. Innumerable cases are cited to illustrate these symptoms or symptom complex. This volume will be found to be of great help both to the clinician and to the student.

P. GUTIERREZ.

Painless Childbirth | Eutocia and | Nitrous Oxid-oxygen Analgesia | by |  
Dr. Carl Henry Davis | [4 lines] | [seal] | Chicago | Forbes & Com-  
pany | 1916 | Cloth, pp. 1-134. Price, \$1.

The first section of the book considers painless childbirth, discussing the departure under conditions of civilization from the ease of delivery said to exist in savagery, and justifies

the use of anesthetics. After sketching the development of anesthetics, it compares, very favorably to the latter, the pharmacology and the results of the practical use of the morphin-scopolomin mixture (known as "twilight sleep") and of nitrous oxide and oxygen in obstetrical work.

The second section, entitled *Eutocia*, considers certain unsatisfactory features of the general conditions that surround the present-day practice of obstetrics in the home and the hospital. A strong case is prepared statistically to show that the maternal death rates are, even with modern knowledge of asepsis, anesthetics, and obstetrical technique, unnecessarily high. This mortality seems largely due to puerperal infections which, under proper conditions, are avoidable.

The third part deals with nitrous oxid-oxygen analgesia in obstetrics. The results of practice in the Presbyterian Hospital of Chicago are analyzed to show the advantages of the analgesia over ordinary labor without anesthetics. The technique of the analgesia is considered, its simplicity, which even permits the patient to administer her own gas, is emphasized, and certain pertinent points for caution are noted.

This unpretentious little monograph seems clearly to point the way to a safe and practicable means of relieving the parturient woman of much of the suffering and nerve shock that ordinarily accompanies the condition. This is in line with the extension of the use of this analgesia in dental and in certain simpler surgical operations.

H. W. W.

**A Handbook | of | Infant Feeding | by | Lawrence T. Royster, M. D. |**  
 [5 lines] | illustrated | St. Louis | C. V. Mosby Company | 1916 | Cloth,  
 pp. 1-144. Price, \$1.25.

Within recent years a good number of handbooks have been written on infant feeding. Many of them have justified their publication, while others seem to be somewhat superfluous. In the little volume under review the author has attempted to furnish the busy practitioner, in "a compact and succinct form," with the essential and practical side of infant feeding, leaving aside all the conflicting and theoretical points of this important subject of pediatrics.

The book contains fifteen chapters and one appendix, which includes the commentaries upon the various constituents of the food; the growth and development and the stools of infants; natural and artificial feeding; the care of premature infants; the digestive disturbance of both breast- and bottle-fed infants;



the feeding of difficult cases; the feeding during the second year; marasmus; infectious diarrhœa; the preparation of formulæ by the percentage method; the composition and preparation of the foods most employed, such as barley water, whey, casein milk of Finkelstein, buttermilk, and batter bread. There is also presented the Harvard classification of gastrointestinal disturbances. Worthy of mention as interesting features of the handbook are the following chapters:

1. The stools of infancy, masterly written by Professor Loretta Morse.

2. The bottle feeding gives valuable hints to modify cows' milk in order to reach "a gradual adaptation of the ingredients to the digestive power of infants."

3. The chapter on the digestive disturbances, in which the author wisely adopts the German division of disturbances in breast-fed and in bottle-fed infants.

4. The chapter on the handling of difficult cases of feeding also must be commended, as presenting very practical advice for the practitioner.

It is to be regretted that the chapter on the treatment of marasmus is so short and rather incomplete.

Altogether the book is a successful attempt to guide the practitioner, especially the American physician, who is very well acquainted with the principles of the percentage method, and to solve the daily problem of conducting the feeding of infants.

JOSÉ ALBERT.

Diagnostic Methods | [6 lines] | by | Herbert Thomas Brooks, A. B., M. D.,  
| [2 lines] | third edition | revised and rewritten | St. Louis | C. V.  
Mosby Company | 1916 | Cloth, pp. 1-96. Price, \$1.

This little book of diagnostic methods is very well adapted to the needs of the medical student, interne, and practicing physician. By referring to it, often many important and essential diagnostic facts may be kept in mind, thereby obtaining more vital data from each patient. Using this handbook as a guide and a large textbook for reference, a decided improvement in the character of medical work performed by the average practitioner should result.

The average textbook on diagnostic methods is altogether too exhaustive for everyday work, and the practicing physician finding it so formidable decides to depend wholly on his native knowledge of this subject. Undoubtedly this handbook will bridge over the gulf and will encourage the physician toward a more complete study of the individual case.

T. F. KEATING.



Candy | Medication | by | Bernard Fantus, M. D. | Professor of Pharmacology and Therapeutics College of | Medicine, University of Illinois, Chicago. | St. Louis | [ornament] | C. V. Mosby Company | 1915 | Cloth, pp. 1-82. Price, \$1.

Candy Medication by Doctor Fantus presents a method for making the administration of medicine less obnoxious to children by using it in the form of candy. He gives formulæ for the use of fifty such medicaments. These fifty pretty well cover the gamut of children's ordinary ailments, and their general administration in the form of candy should be hailed as a distinct advance in robbing childhood of its dread of obnoxious medicine.

The book is well written and free from any serious objectionable features. It is a distinct addition to the literature of medicine dispensing and deserves the favor of the physician and pharmacist. The dose in most cases is small, being usually about one tenth the size of the average dose recommended by the United States Pharmacopœia. However, as the author states, the smallness of the dose is an advantage, since it necessitates frequent administration which is a good principle in practice, owing to the greater activity of the vital processes of the child.

H. C. BRILL.

Post-Mortem | Examinations | by | William S. Wadsworth, M. D. | Coroner's physician of Philadelphia | with 304 original | illustrations | Philadelphia and London | W. B. Saunders Company | 1916 | Cloth, pp. 1-598. Price, \$6 net; half-morocco, \$7.50 net.

Wadsworth's book contains much that is praiseworthy and not a little that may be criticized. The scope of a post-mortem examination is so great that it is a matter of some difficulty to decide what shall be included in, and what excluded from, a book devoted to the subject. The author is correct in his idea that a post-mortem operator should have a broad knowledge of the medical sciences, but it is manifestly impossible to inclose an encyclopædia of these sciences between the covers of one book. That part of the author's work that is devoted to description of technique is for the most part excellent, many original observations have been recorded, and the illustrations are well selected and beautifully executed. The intense personal element which everywhere pervades the text does not materially add to the value of a book which from its very nature should derive its greatest circulation among those without a large mortuary experience. A solved problem appears to have presented fewer difficulties than before its solution was reached, and post-mortem revelations are too common an incident to call for diatribes against every one but the post-mortem operator. Charity toward

men in the clinical branches would be more frequent if the "post-mortem operator" could see more of the doctor's cases that do not come into his hands. At any rate, the book is one which cannot be unconditionally recommended for medical students.

The publishers deserve very great credit for the appearance of the book.

B. C. C.

Pellagra | an American problem | by | George M. Niles, M. D. | [4 lines]  
| second edition | illustrated | Philadelphia and London | W. B.  
Saunders Company | 1916 | Cloth, pp. 1-261. Price, \$3 net.

This book on pellagra is the second edition of one of the best American treatises on a condition which, previous to 1907 and 1908, was seldom recognized in the United States. Since that time it has increased and spread so alarmingly that it has become one of the serious American problems. Under these conditions, when much of the medical profession is unsatisfactorily informed as to the disease, a book such as that under review is particularly of value. It is intended for the physician and is written by a physician who has evidently been embarrassed in his efforts to keep in touch with all features of so many-sided a question as pellagra.

The work takes up the historic and other considerations; various phases of the etiology controversy are detailed; the symptomatology and clinical course are discussed and followed by an illustrative chapter of case reports; the pathology and morbid anatomy are briefly considered; the diagnosis, treatment, and prophylaxis are thoroughly discussed; and certain reports of animal experiments are added as a final chapter.

The book as a whole is written in a somewhat chatty style which often makes for pleasant reading, though occasionally it is carried so far as to detract from the seriousness of the work. The sections of the book which deal with phases familiar to the author exhibit a confidence and authority in contrast to other sections covering less familiar territory. The discussion of such subjects as diagnosis and treatment are valuable contributions in which the work of others is considered judicially, from the standpoint of much experience. Upon other features, however, the author sometimes contents himself with more or less extensive quotations and excerpts, outlining various hypotheses which are often antagonistic with little or no expression of opinion or personal experience.

The various theories of etiology are outlined, and a preponderance of evidence is shown to be in favor of the idea that pellagra

is a disease of dietary deficiency, in which the author believes that altered corn plays a leading rôle. In the discussion of the nutritive value of corn, it would seem that recent work on the chemistry of corn as a food has not been noted. Further, presumably because it was sent to press too early, Goldberger's last report, based on his Mississippi experiments, was not included. This is probably well, for the early reports of this work are inconclusive, and the author already lays sufficient emphasis on the dietetic hypotheses.

A separate, "somewhat supplementary chapter," which deals with "some recent experiments on animals, and deductions therefrom" is confined to two papers, one by Lavinder and the other by Anderson and Goldberger. It is now deficient as a summary of the subject, as it is evident that no attempt has been made to bring it to date in the new edition. Thus the latter article is still spoken of as "a recent bulletin," and the subsequent and apparently successful inoculation experiments of Harris, of New Orleans (1913), which at least are worthy of consideration, are ignored.

Occasionally there appear instances of careless editing, as where, in a quotation from a "recent" article (which was published in 1910), the word "root" is missing from "the posterior of the spinal nerves" (p. 159), or the word "flourescent" for "fluorescent" (p. 239) in a discussion of food substances. More noticeable is the evidence of hasty revision. Phrases such as "a recent case," a case seen "several months ago," or an advertisement which "the daily papers have recently carried" are inappropriate in a work intended to run for more than one edition. To speak of a personal report from a man "who has recently held four postmortems" (p. 163) and then add (p. 165) that "Since the first edition of this book was published \* \* \* has performed," is, to say the least, unusual.

Such faults of style and revision as those suggested cannot but prejudice the reception of any work, and it is particularly unfortunate in this case as there is, in the body of the book, much material of value to the physician whose problem is the recognition and treatment of the disease.

H. W. W.





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CONGENITAL BILATERAL ABSENCE OF KIDNEYS IN A  
140-MILLIMETER PIG EMBRYO <sup>1</sup>

By ARTURO GARCIA

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University of the Philippines)*

INTRODUCTION

ONE PLATE AND 3 TEXT FIGURES

A review of the literature on renal malformations and maldevelopments has demonstrated the fact that, while unilateral congenital absence and maldevelopment of kidneys have been frequently recorded in the human and lower animals, bilateral cases, though observed with comparative frequency in embryos, have been reported but once or twice in older human beings.

There is naturally an obvious reason for this difference in frequency of occurrence. While unilateral congenital absence or defective conditions of kidneys are compatible with extra-uterine life, when both organs are wanting, life is hardly conceivable; and, of course, cases that go on to adult life are much more apt to be observed and reported than those conditions which might exist only in utero. It is also for this reason that congenital unilateral renal defects acquire much greater importance to the clinician and to the surgeon than similar bilateral defects. It is now a surgical routine for men doing kidney work to keep in mind congenital unilateral absence of kidney. Nephrectomy in such cases would invariably be fatal.

As to absence of both kidneys, however, though it lacks the clinical importance of unilateral renal aplasia or congenital absence of one kidney, its developmental significance is of such interest to students of embryology that a careful report of all such cases would seem warranted. It is for this account that

<sup>1</sup>Received for publication August, 1916.

the present case is reported, especially since the rest of the urogenital tract seemed to be apparently normal, differing in this respect from many of the cases so far recorded.

Whereas up to date some three hundred cases of unilateral absence of kidneys have been reported, a careful search of the literature on hand shows only the following men reporting instances of bilateral renal absences.

Gerard<sup>(4)</sup> cites Moulon, Beclard, Meyer de Bonn, Chausier, Pignée, and Dubierre as having reported cases of congenital absence of both kidneys. In the case of Moulon, a girl about fourteen years of age, both kidneys and ureters were absent, while the umbilicus opened in the region of the mons veneris and constantly exuded a liquid resembling urine. Ahlfeld,<sup>(1)</sup> English,<sup>(3)</sup> Hauch,<sup>(6)</sup> Rud Bayer,<sup>(7)</sup> and Zaufal<sup>(9)</sup> also reported cases of bilateral renal absence. Greenfield<sup>(5)</sup> describes two cases of a similar defect, in one of which the vas depreus and the testicles also were absent. Schaffaer<sup>(8)</sup> mentions the case of a 7-month-old human foetus without any traces of kidneys, and Coen,<sup>(2)</sup> according to Ballowitz, in a study of thirty-three monstrosities, found fifteen cases of total absence of kidneys, in which the adrenals were larger than normal.

There are probably more cases reported than those mentioned, but with the literature available it has been impossible for us to add any more or even to verify some of the above reports.

#### MATERIAL USED

The present specimen was obtained while on a trip to the slaughterhouse of the city to inject kidneys of pig embryos. The case is a female pig embryo, 140 millimeters long, from a litter of five apparently perfectly normal specimens of approximately equal size.

#### ANATOMY AND HISTOLOGY

In external appearance and on careful examination of the thoracic and abdominal cavities, this specimen appears normal. Our attention was called to the absence of the kidneys only after looking in vain for them to make our injection. The genitalia also appear normal; the ovaries measure 5.5 by 3 by 3 millimeters. The oviducts and uterus are in every respect identical with those of pigs of the same size.

In the urinary apparatus the bladder is well developed, measuring 12 by 4 by 4 millimeters and ending in a well-formed

urachus. The two hypogastric arteries are also well defined and normally located. The kidneys are absent, and no traces of what might be taken for rudiments of these organs can be found in the neighborhood of their normal position. The two ureters are well developed, and their orifices at the trigone are patent. The right ureter, which appears slightly larger and longer than the left, begins from the right inferolateral side of the bladder, passes in an upward and lateral direction for about 9 millimeters, then continues upward in a wavy course over the quadratus lumborum for about 16 millimeters, and ends in a funnel-shaped expansion which is lost in the retroperitoneal fascia in this region. The left ureter, slightly smaller in diameter than the right, is decidedly shorter and lacks an abdominal portion. Traced from the lower inner side of the bladder, it passes in a lateral direction to the back of the pelvis with a slight concavity upward for a distance of 6.5 millimeters and ends in a thin membrane attached to the posterior pelvic wall.

Occupying apparently normal positions, the adrenals appear as two large, more or less pyramidal masses which are about three times as large as those of pig embryos of approximately the same size.

Retroperitoneally, over the right quadratus lumborum, and on a level with and lateral to the expanded upper end of the right ureter, a small mass of glandularlike tissue of irregular shape is found. This was removed, marked "G," and imbedded for histological examination. Two similar bodies were seen on the right lateral portion of the posterior abdominal wall, one immediately above and the other to the mesial side of the right ovary; both were removed for embedding and marked "D" and "H," respectively. On the left side two similar masses were also seen, one of triangular shape a few millimeters lateral to the left ovary, and the other oval in shape, immediately below it. These were embedded as "B" and "A" respectively. No other masses of tissue which might be looked upon as suspicious traces of kidney were seen in this region (fig. 1).

The abdomens of other pig embryos approximately of the same size were opened and studied for comparison.

In a pig embryo 120 millimeters in length the right kidney measured 15 by 7.5 by 7 millimeters; and the left, 15.5 by 7.5 by 6.5 millimeters. Both were situated on the posterolateral wall of the abdomen and were so prominent as to be the first things noticed on lifting up the coils of the intestine. Capping both organs were the two triangular adrenals which were about one third as large as those found in our kidneyless specimen. The



right ureter was about as large in diameter as the corresponding ureter of the pig in question. It started from the pelvis of the kidney and followed a similar course down to the bladder. The left ureter was also well developed; it began from the pelvis of the left kidney, passed downward parallel with the hypogastric artery for about 15 millimeters behind the broad ligament of the uterus, and then made a turn medially for about 5 millimeters and buried itself in the lower pole of the bladder. The ovaries were both normally placed, and the right measured 4

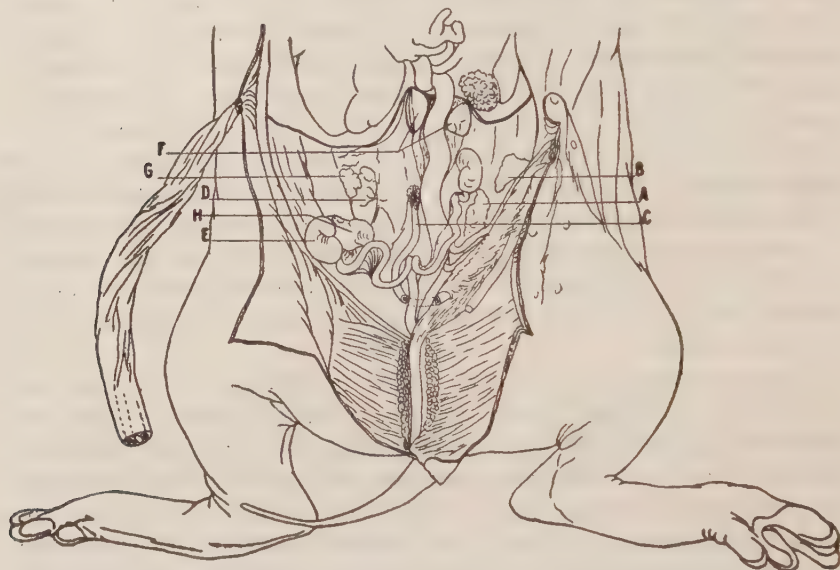


FIG. 1. Schematic drawing of abdominal cavity with intestinal coils reflected.  $\times 3$ .

by 2.75 by 2 millimeters. The rest of the abdominal organs appeared normal and corresponded identically with those in the specimen.

Owing probably to prolonged fixation in formalin there was a generalized contraction of the tissues of our specimen. All the sections were stained with Delafield's hæmatoxylin and eosin.

Sections from the right ovary showed a richly vascular medulla and a cortex studded irregularly with primitive follicles, many of which contained a well-formed ovum. Between these follicles, and embedded in a reticulum of young connective tissue, cords of Pflüger were plainly seen. The stroma of both the medulla and the cortex was composed of young connective tissue cells covered by a germinal epithelium which was torn in many places. The outer end of the right oviduct was also sectioned and stained. It showed a well-formed lumen lined with pseu-



dostratified, columnar, ciliated epithelium surrounded by a layer of vascular, young connective tissue in many places richly infiltrated by lymphocytes. Between the section of the oviduct and the rete ovarii, in the mass of tissue which represents the broad ligament, several distinct mesonephric tubules, which are part of the parovarium, were seen.

The last 6 millimeters of the upper end of the right ureter was embedded for examination. Sections from the lower end of this piece of ureter showed a well-defined oval lumen lined with transitional epithelium, the cells of which for the most part were ovoid in shape. Their nuclei stained faintly and were placed near the cell base. Surrounding this epithelium, two distinct layers of tissue were seen; the inner coat, slightly the thicker

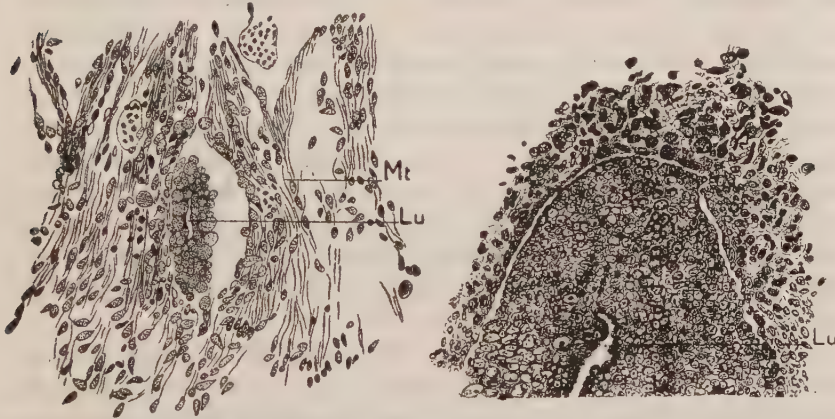


FIG. 2. Section of upper end of ureter (right). FIG. 3. Section of lower end of right ureter.

of the two, appeared to be made up of young connective tissue cells which were of irregular shape and showed collections of lymphoid tissue in many places. The outer layer was made up of fusiform cells more loosely arranged. In this coat numerous blood vessels were seen. Sections taken higher up had smaller lumen lined with transitional epithelium, the cells of which appeared better stained and their outlines more distinct. The surrounding connective tissue layers were looser in texture, the cells becoming more starshaped and approaching more the character of mesenchymal cells. Higher up the ureter breaks up into two or three solid cords of epithelioid cells surrounded by loose mesenchymatous tissue, which now exhibits here and there collections of lymphoidlike cells resembling to a certain extent small lymph glands. In no section of the entire 6 millimeters of ureter could we find any tissues which might in any way suggest the slightest structure of the blastemal cap, which is always present in normal cases (figs. 2 and 3).

Sections from both adrenals showed a well-defined capsule of white fibrous connective tissue. Between this capsule and the glandular substance is a narrow strip of vascularized young connective tissue, in which irregularly shaped masses of cortical and medullary cells were to be seen. The cortex of the gland appeared as a narrow band of cellular elements surrounding the medulla. This cortex is composed of a vascular reticulum in the meshes of which cortical cells, polygonal in shape, are arranged in vertical cords distinctly revealing the beginning of the zonas glomerulosa and fasciculata. Toward the periphery these were granular and showed the characteristic fat granules observed in adult cells; their nuclei, ovoid and round, filled from one fourth to one half of the cell body and for the most part were centrally located. Toward the center these cells gradually merged with those of the medulla. The medulla, comparatively more abundant than in adult adrenals, was separated at places from the cortex by a large blood vessel. There is a distinct vascular reticulum, the mesh spaces of which were filled with irregularly shaped medullary cells, with large ovoid nuclei placed centrally and almost filling the entire body of the cells. These cellular elements were arranged in irregular branching masses not unlike those of the parathyroid glands. The blood vessels were for the most part thin-walled with a distinct endothelial lining.

Sections from masses "A," "B," "G," and "H" were apparently identical. There was a thin fibrous connective tissue capsule with adipose deposits at places, within which was a delicate, vascularized connective tissue reticulum. The mesh spaces of this reticulum were lined and contained large flattened and irregularly ovoid cells with round nuclei centrally placed and filling about one fourth or one half of the body of the cells. Masses and isolated cells of lymphoid nature were to be seen. These cellular elements were seen to be separated from the lining of the sinuses and from the reticulum by narrow spaces. In some sections, especially in those of "H," small eosinophilic cells were also observed. These cellular bodies were small lymph glands.

Sections from "D" were apparently made up of vascularized embryonic connective tissue.

#### EMBRYOLOGY

In considering the embryology of the urinary excretory system, especially in amniotes, we are at once impressed with the fact that we are dealing not with a gradual development of one

single organ which is laid down and gradually brought up to its definitive completion, but to a saltatory development of three different organs, the pronephros, the mesonephros, and the metanephros, which are developed and formed in succession, each being apportioned and adapted to a definite period of one entire development. Of these organs the pronephros and the mesonephros are merely provisional kidneys; their activities become superfluous and partially or totally disappear and are eventually supplanted by the last and third organ, the metanephros, which becomes the adult organ of urinary excretion.

The anlage of these three organs is the same; the cells of the intermediate mass of mesoderm of the embryo, from the cephalic portion of which develops the pronephros, from the middle zone the mesonephros, and from the caudal end the metanephros.

In the present case the pronephros has evidently undergone its full development and degeneration, leaving only behind its primary collecting duct from which the two ureters have apparently normally developed.

That the mesonephros has also undergone its normal course of development and degeneration is evidenced by the presence of distinct mesonephric tubules in the region of the rete ovarii forming the parovarium, the normally formed uterus and oviduct, and the two well-developed ureters, which are all derivatives of the mesonephros.

In the case of the metanephros, the ureter in the right side has apparently developed upward to its normal length, and its upper extremity has started to expand into a funnel-shaped primitive pelvis and to divide into several primary collecting tubules. No evidences can be seen, however, of a blastemal cap accompanying it in this upward growth. The left ureter has apparently become arrested at the brim of the pelvis, becoming entangled there in the retroperitoneal mesenchyma.

Whether these ureters were accompanied by blastemal tissue from the nephrogenic cord which might have undergone complete regression or not is a question which cannot be fully determined with the present findings.

#### SUMMARY AND CONCLUSIONS

The review of the literature has shown the comparative rarity of congenital absence of kidneys, especially in man. Only a few cases have so far been reported, one or two of which are inconceivable.



The absence of the two kidneys, though embryologically interesting, lacks the clinical importance of the unilateral cases, owing to the fact that it is incompatible with life.

The specimen studied in the present case was an apparently normally developed embryo in no outward point different from the others of the same litter.

There are apparently no other abnormalities found in the rest of the urinary apparatus aside from the absence of both kidneys and the short and maldeveloped left ureter. Both ureters were normally placed, and both ended in expanded extremities, which were lost in the surrounding fascia.

The genital tract appeared normal as compared with embryos of approximately the same size.

The adrenals were larger than normal and appeared rather advanced in differentiation.

There were no traces of tissues in the region of the nephrogenic cord, which on microscopic study might suggest the least appearance of renal blastema. The four or five glandular collections, which might be regarded as suspicious, were demonstrated to be lymphoid bodies and masses of mesenchymatous tissue without signs of tubular formation whatsoever.

From the present case the following conclusions may be drawn:

1. In the case of the right ureter the absence of kidney is probably due to a failure of the nephrogenic blastemal cap to appear or to develop, the upper expanded end of this ureter having undergone tubular divisions preparatory to the formation of the medulla of the kidney.

2. The left ureter may have been arrested in its growth cephalad and become entangled with the mesenchyma of the pelvis, thus failing to reach the nephrogenic cord and meeting the blastemal substance which might have existed and undergone regression.

3. From the apparently normal development in general of this specimen as compared with the others of the same litter it is probable that kidneys are not essential for proper growth intra utero.

4. The adrenals said to be generally larger in renal absence as reported by Coen and others is also confirmed in this specimen.

This work has been done under the immediate supervision of Dr. E. S. Ruth, to whom I wish to express my sincere thanks for kind and very helpful suggestions. I also wish to thank Dr. B. C. Crowell for valuable help in the microscopical study of the sections.



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## ILLUSTRATIONS

[From drawings by Juan Castro.]

PLATE I. Female pig embryo, 14 centimeters long.  $\times 3$ .

### TEXT FIGURES

- FIG. 1. Schematic drawing of abdominal cavity with intestinal coils reflected.  $\times 3$ . *A, B, G, H*=lymphoid collections; *F*=adrenals; *D*=mesenchymatous body; *E*=right ovary; *C*=upper end of right ureter.
2. Section of upper end of ureter (right). *Lu*=lumen of ureter; *Mt*=mesenchymatous tissue. Objective 6; ocular 4, Leitz.
3. Section of lower end of right ureter. *Lu*=lumen of ureter. Objective b; ocular 4, Leitz.





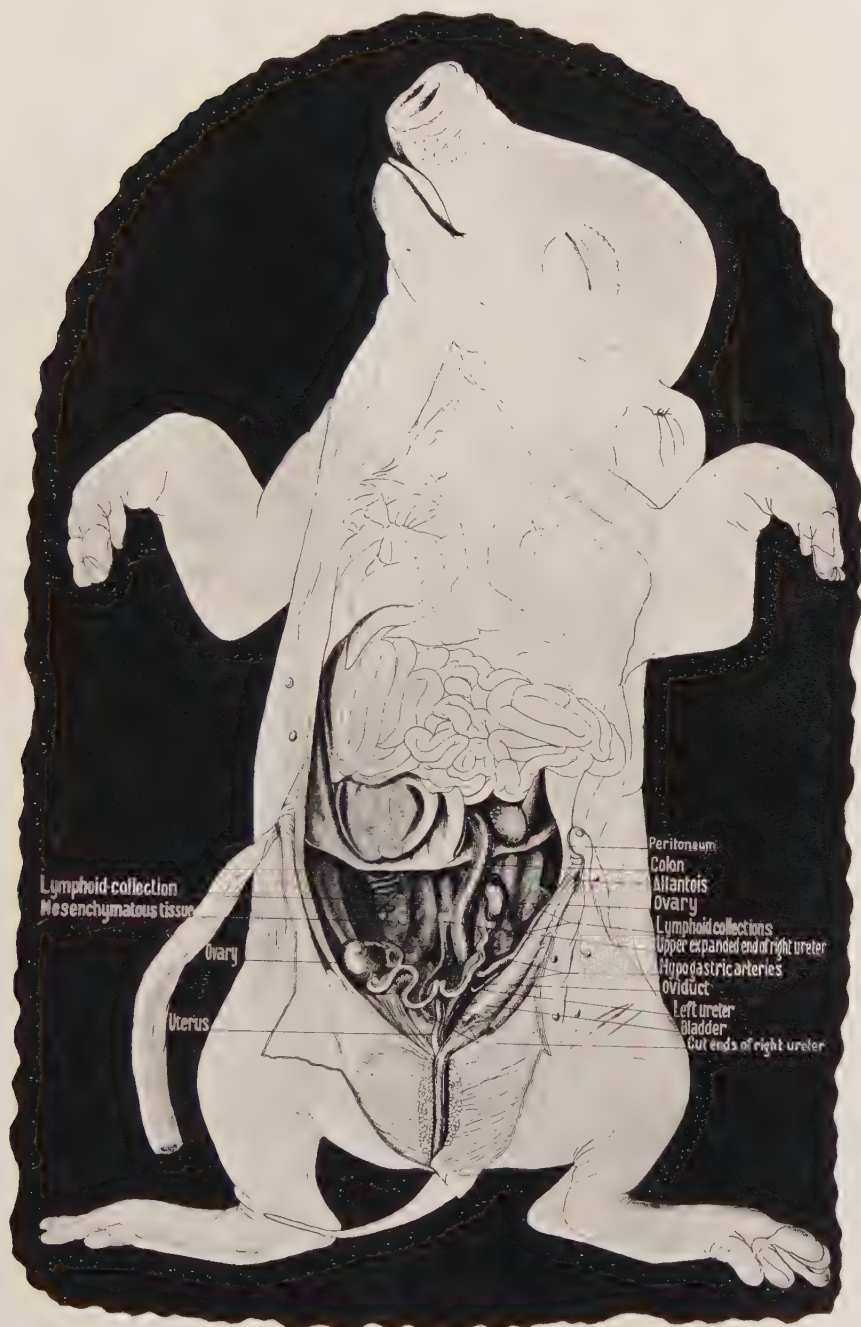


PLATE I. FEMALE PIG EMBRYO, 14 CENTIMETERS LONG.



## POISONING BY *ILlicium religiosum* Siebold<sup>1</sup>

By LUIS E. GUERRERO, D. DE LA PAZ, and ALFREDO L. GUERRERO

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Poisoning from the use of parts of plants does not occur very frequently in the Philippine Islands. We are not aware of any official statistics or publication on this subject; neither are we aware that the various species of poisonous plants which abound in the Philippine flora have been defined and enumerated. But in reality poisoning from the use of these plants in the Islands is by no means as rare as one would be led to believe after consulting the literature on the subject. We know from reliable sources that in Manila and in the provinces have occurred intentional and accidental cases of poisoning by *namí* (*Dioscorea daemona*), *maná* (*Jatropha multifida*), *suma* (*Anamirta cocculus*), *salagó* (*Wikstroemia ovata*), *katbalonga* (*Strychnos ignatii*), *talampunay* (*Datura alba*), *tuba* (*Jatropha curcus*), and other plants of still undetermined status.

### REPORT OF FOUR CASES OF POISONING BY *ILlicium religiosum* Siebold WHICH OCCURRED IN MANILA

Recently we have had occasion to observe four cases of poisoning from the use of a decoction of *sanki*, the fruit of *Illicium religiosum* Siebold. Since the symptomatology and therapy of this poisoning are not well defined, and no mention of them is made in the textbooks of tropical medicine in current use except that by Grall and Clarac<sup>(1)</sup> and by Mense,<sup>(2)</sup> the report of these cases might be interesting and of some clinical value.

#### CASE 1

Mr. N. N., 50 years old, believed through hearsay that a decoction of *sanki* would be a good stimulant, stomachic, and excellent remedy for cholera. On October 24, 1914, at 3 o'clock in the afternoon, he took two cups of a strong decoction of *sanki* (exact quantity of the poison taken undetermined). At 4 o'clock, that is, one hour later, repeated vomitings occurred. These were accompanied by bowel evacuation of large quantities of fluids, cramp in the calf muscles, profuse perspiration, and intense thirst. The patient complained of severe headache, vertigo, and extreme weakness, especially in the lower extremities. At 4.30 he became restless, tossed in bed, and after a

<sup>1</sup> Received for publication September, 1916.

few minutes was seized by clonic and tonic convulsions. During the attack of convulsions the face was congested and expressed fear, the respiration stopped, the eyeballs diverged, the head was retracted toward the back, and the trunk and the extremities were rigid and extended, giving now and then slight convulsive movements. After this single attack the patient was exhausted and markedly prostrated, but was conscious and showed no sign of disturbance of mental equilibrium. The pulse was large and strong at the beginning, but became rapid and thready afterward; there was dyspnoea, and the urine was scanty and highly colored. The presence of albuminuria was not determined.

With the belief that we were dealing with a case of cholera, the patient was given 500 cubic centimeters of physiological salt solution and 1 cubic centimeter of camphorated oil, injected hypodermically, and hot stimulating drinks. Five hours after the appearance of the first symptom the patient was distinctively improved and rapidly recovered.

#### CASES 2 AND 3

These cases are the daughter and the cook of Mr. N. N. (case 1), each of whom took a very small quantity of a decoction of sanki on the advice of the latter. Emesis appeared shortly after taking the poison, and the patients complained of no disturbances other than temporary dizziness. The mildness of the symptoms in these two cases was undoubtedly due to the early onset of vomiting and presumably to the small quantity of the poison ingested.

#### CASE 4

This case was a woman who three days after labor was given by her mother a cup of sanki decoction as a stimulant. Again the exact quantity of the poison ingested unfortunately could not be determined. Four hours after taking the poison the patient developed the following symptoms: Vomiting, cephalalgia, cramps in the legs, convulsions resembling those in case 1, profuse sweating, and oliguria. Diarrhoea, restlessness except during the convulsions, and marked prostration did not occur. The administration of chloral hydrate caused the convulsions to disappear; the patient recovered.

Dr. Tee Han Kee has very kindly communicated to us privately that more than twenty cases of poisoning by *Illicium religiosum* have come under his observation. These cases were all Chinese residing in Manila, who used the poison in committing suicide or for the purpose of relieving tympanites and various ailments, such as hydrocele and anasarca, which they attribute to cold.



He has also kindly sent to us a detailed description of three more cases in Chinese girls recently attended by him. All the girls developed convulsions, and one of them died.

#### ETIOLOGY OF POISONING BY *ILlicium RELIGIOSUM*

The fruit of *Illicium religiosum* is not used as a condiment nor as a medicine. Under the name of *Illicium anisatum* it is employed in some towns of the Orient in small, indefinite quantities to flavor certain native hodgepodes. The Chinese in Manila acknowledge that the fruit of this plant is highly poisonous; yet they do not distinguish it from *Illicium anisatum*, and they call indiscriminately these two species of *Illicium* fruits *pue-kag* (star of eight rays). They consider both species poisonous. Eykman<sup>(3)</sup> and Langaard<sup>(4)</sup> established experimentally the toxicity of *Illicium religiosum*, but so far as we know the relative toxicity of *Illicium anisatum* and *Illicium religiosum* is not yet determined. According to our unpublished experiments carried on in the department of pharmacology, College of Medicine and Surgery, University of the Philippines, in collaboration with Miss Felisa Nicolas, the fruit of *Illicium religiosum* is fourteen times more toxic than that of *Illicium anisatum* when kittens are used as the test objects. The fruit of *Illicium religiosum*, in the proportion of 0.25 gram per kilogram of body weight, was injected hypodermically in the form of an infusion. It is a sure minimal fatal dose to these animals. *Illicium anisatum*, on the other hand, is extensively used by the laity as a stimulant, stomachic, and carminative. The Filipinos use it in the preparation of various dishes and to flavor a drink known as *tahu* or *salabat*, which is nothing more than a sweetened decoction of ginger. *Tahu* is popular in many provinces of Luzon, and the poor people drink it in place of tea and coffee. At the present time *Illicium religiosum* is the only variety which is sold in the groceries of Manila, while *Illicium anisatum* is obtainable only from the drug stores. It is, therefore, not strange that cases of poisoning by *Illicium religiosum* should occur in Manila. It is highly probable that it is the materies morbi of the so-called *colerinas* which have been observed to follow the ingestion of *salabat* flavored with *sanki* in some towns of the Philippines. On account of the cheapness of *Illicium religiosum*, it is frequently substituted for *Illicium anisatum*. This substitution has been a cause of the poisoning recorded in Holland, in France, and in other countries and is illustrated by the cases observed by Montel<sup>(5)</sup> in three Europeans in Conchin China. These cases present

different stages of the poisoning, and we consider it interesting to reproduce them in detail.

## CASE 1

Mr. O. had been taking infusions of *Illicium anisatum* for diarrhœa. Having used his provision, he ordered more *Illicium anisatum* from a drug store, on October 6, but his servant went to a Hindu grocery and obtained *Illicium religiosum*. Mr. O. took of it in the form of an infusion about 7.30 of the same morning. At 9 o'clock he began to have repeated vomitings, profuse diarrhœa, and cramps in the legs.

Convulsions set in at 11 o'clock. The patient became suddenly restless and unconscious after giving a loud cry. During the convulsion the breathing stopped, the eyeballs rotated laterally, the face expressed fear, the muscles of the trunk contracted, the head was retracted toward the back, the forearms were flexed, the hands were clinched, and the limbs were rigid, giving occasional jerks. This paroxysm lasted one or two minutes and resembled that of the classical idiopathic epilepsy. After ten minutes consciousness suddenly returned, the respiration was quite, and the patient complained of cramps in the legs.

Between 11 o'clock in the forenoon and 9 o'clock in the evening this convulsive paroxysm was repeated five times. The patient, at the interparoxysmal periods, was exhausted, complained of severe headache, was restless in bed, and assumed a Z-position. He was thirsty and drank a large quantity of Vichy water, which he vomited. The vomiting was frequent; it lasted till 3 o'clock in the afternoon. There were anuria, carphology, and clouding of consciousness; the patient was not able to recognize the persons surrounding him. The pulse was 120 beats per minute and was small.

The patient was given 0.5 gram of caffeine, 1 cubic centimeter of camphorated oil injected hypodermically, and a little antipyrine which seemed to alleviate the headache. He was also injected with physiological salt solution, 500 cubic centimeters at noon and another 500 cubic centimeters at 10 o'clock in the evening. After the second injection the general condition was improved and the convulsions did not return. At night he passed urine and slept.

On the morning of October 7 he urinated again and slept a little. The headache disappeared, but there were still mental dullness and physical weakness. In the afternoon the temperature was 38.7° C., the pulse was 106 beats per minute, and there were asthenia and somnolence. The temperature rose

to 40° C. at 10 o'clock in the evening, but later it subsided. The patient urinated seven times during the night.

On October 8 the general condition of the patient was excellent. There was only slight mental depression, which gradually disappeared. The patient recovered completely and had no recollection of anything that had happened after the ingestion of the poison. He did not remember the approximate quantity of the *Illicium religiosum* which he had taken.

Cases 2 and 3 were Mr. and Mrs. B., who one afternoon took an infusion of *Illicium religiosum*. Mrs. B. drank a smaller portion of the poison. She had early vomiting and profuse diarrhoea. Vomiting did not appear in Mr. B. until one hour later, and the vomitus was less abundant. They developed convulsions resembling those described in the case of Mr. O. Mrs. B. recovered, but her husband, in addition to the convulsions, suffered from anuria, hallucination with tendency to commit violence, and marked paresis of the lower limbs. On the third day he showed considerable improvement, but afterward, anuria, restlessness, insomnia alternating with depression, cyanosis, cold extremities, and oedema in the calf muscles manifested themselves, and on the eighth day the patient died of asphyxia. Post-mortem examination revealed congestion of the alimentary tract and enlarged, congested kidneys.

Geerts(6) reported six cases of poisoning by *Illicium religiosum*. These cases occurred in Kanagawa, near Yokohama, Japan, and resulted from the substitution of the sesame oil by the fixed oil of *Illicium religiosum* in the preparation of food. In one of these cases death occurred. Other cases of poisoning arising from the substitution of *Illicium religiosum* for *Illicium anisatum* have also been reported by Planchon and Dreyer,(7) Vogl,(8) Barral,(9) Delotte,(10) and Lamarque.(11)

In addition to these cases Eykman mentioned some cases of poisoning which took place at Leeuwarden in the Netherlands, which were caused by fruits which corresponded in botanical description and pharmacological action to the fruits of *Illicium religiosum*. He further mentioned five cases of poisoning in five children at Uyeno Park, Tokyo. These children were found playing at the park, foaming at the mouth and violently convulsed. The opinion that these were cases of poisoning by *Illicium religiosum* was based upon the occurrence of a number of fruit-bearing specimens of the plant in Uyeno Park in the neighborhood of the place where the children were playing and the discovery of some seeds of *Illicium religiosum* in the sleeves of the dresses, in the vomitus of one child, and in the fæces



of another. Three of the children died. There were observed contracted pupil in one, mydriasis and bloody vomitus in three cases, and foaming at the mouth, which was a prominent symptom in our experiments on cats. Otherwise the symptomatology was, in general, similar to that described by Montel and by us.

#### SYMPTOMATOLOGY AND DIAGNOSIS OF POISONING BY ILICIIUM RELIGIOSUM

According to these cases the following symptoms may occur in cases of poisoning by *Illicium religiosum*: Foaming at the mouth, repeated vomiting, diarrhoea, thirst, unconsciousness, clonic and tonic convulsions, cramps in the legs, cephalalgia, mental disturbances, insomnia, profuse sweating, oliguria or anuria, large strong pulse giving place to small rapid pulse, cold extremities, contracted or dilated pupils, paresis of the lower limbs, and exhaustion. The vomitus may be streaked with blood. During the convulsions the pupils as a rule dilate, the eyeballs bulge, the head is retracted toward the back, the respiration stops, and the face is cyanotic. According to Burdin (12) paresis of the limbs was the most prominent symptom in all the cases which came under his observation. Ferraud (13) claims that it can be reproduced exclusively by varying the dose of *Illicium religiosum* given to experimental animals. This we have not yet studied carefully. In severe or fatal cases of poisoning in man, repeated vomiting, diarrhoea, clonic and tonic convulsions which appear in paroxysm, cramps in the legs, and retraction of the head appear to be the most conspicuous symptoms. Cholera, strychnine poisoning, tetanus, and perhaps cerebrospinal meningitis may be confounded with *Illicium religiosum* poisoning; but the history that the above symptoms followed the ingestion of the *Illicium*, and its presence in and about the house and particularly in the vomitus and bowel evacuation of the patient, should readily clear the difficulty in establishing a definite diagnosis.

#### IDENTIFICATION OF ILICIIUM RELIGIOSUM

The fruit of *Illicium religiosum*, according to G. Planchon and E. Collin,<sup>(14)</sup> is generally two thirds the size of that of *Illicium anisatum*. The former seldom develops the full number of carpels, and for this reason the rays formed by them are rarely regular. The superior border of the carpel is never a horizontal line, but it turns upward at the end, giving the individual ray the form of a claw. The depression on the



lateral surface produced by the adjacent carpel is generally conical in outline, while in *Illicium anisatum* it is semiellipsoid. The seed of *Illicium religiosum* is a trifle smaller and presents an obtuse apex, due to the development of the raphe. When the fruit is powdered, it gives off the odor of cubeb or laurel and not the unmistakable anis odor of *Illicium anisatum*. Vogl (15) states that the sclerenchyma layer lining the endocarp at the ventral suture is softer and is composed of cells whose walls are in general thinner than in *Illicium anisatum*. He also states that the cells of the endocarp at this level are shorter than in the last species. The content of these cells turns brownish black on coming in contact with a hot solution of potassium hydroxide. Godfrin (16) emphasizes the fact that the differential characteristics are to be sought for in the seeds. He claims that the seed of *Illicium anisatum* contains only parenchymatous cells under the external sclerenchyma layer of its envelope, while *Illicium religiosum* contains sclerenchyma cells in the corresponding part of the seed. Rud. Pfister (17) bases the differentiation on the form and size of the grains of the aleurone contained in the seed. They are generally ovoid, and the globules contained in them are very small and appear like rounded dots in *Illicium religiosum*; they are larger and irregularly sinuous or tuberos in *Illicium anisatum*. Holmes (18) claims that the two species of *Illicium* react differently to blue litmus paper; *Illicium religiosum* when moistened turns it bright red, *Illicium anisatum* only pale red. Montel summarizes in the following table the distinguishing characters of the two species:

TABLE I.—Differential characters of the fruits of *Illicium religiosum* and of *Illicium anisatum*.

Differential characters.	<i>Illicium religiosum</i> .	<i>Illicium anisatum</i> .
Odor .....	Suggests the odor of pepper and of yew-tree.	Of anise.
Seed .....	Slightly ovoid .....	Flattened.
Follicles .....	Slender, the surface rough, the ends pointed, curved in the form of a claw.	Stout, woody, the surface less rough, ends obtuse.
Axis .....	Tapers gradually upward, terminates slightly below the extremity of the borders of carpels, and appears to incline somewhat toward the lower part of the latter.	Slightly truncated above, forming a disc in to which the fruits are inserted.
Sclerenchyma cells of the peduncle and of the axis.	Few, small, rounded or elliptical.	Numerous, large, and their wall thick.
Anetol .....	Absent .....	Considerable.

According to Montel the easiest method of differentiating one *Illicium* from the other is to boil a follicle of the fruit with one or two cubic centimeters of alcohol in a test tube. With *Illicium anisatum* the addition of water to the alcoholic solution causes the formation of an opalescent precipitate resembling that of absinth of commerce, due to the precipitation of anetol. With *Illicium religiosum* the alcoholic solution remains limpid, but on evaporation gives beautiful crystals of shikimic acid; *Illicium anisatum* gives a very little quantity of small crystals and sometimes none at all. In case the fruit of *Illicium religiosum* were taken in fine powder, Collin (19) advises the microscopic examination of the vomitus or the content of the stomach for fragments of the epicarp and of the endocarp and for the external envelope of the seminal integument. The distinctive characteristics of these parts of the fruit are the striated cuticle lining the epicarp, the special configuration of the sclerenchyma cells of the endocarp, and the highly sinuous sclerenchyma cells of the external envelope of the seminal integument, which also contains crystals in its internal envelope.

Most of the properties of the two species of *Illicium* given above are of value only to the experts. It is self-evident that the laymen, who daily use the fruit of *Illicium anisatum* in the preparation of food and who, on account of their social condition, are less protected against all sorts of adulteration in spite of the Pure Food and Drug Law now in force, require the character which is decisive and is easily detected. Eykman noted that the fruit of *Illicium religiosum* may sometimes simulate the anislike odor and taste of *Illicium anisatum*, but its usual taste and odor of cubeb and laurel are in our opinion the most practical signs for laymen to use in the differentiation of one variety from the other. The detection of the odor and taste is facilitated by triturating or merely crushing one or two pods of the fruit.

Holmes (20) gives the differential properties of the fruits of the various species of *Illicium* as is shown in Table II.

TABLE II.—Differential properties of the fruits of various species of *Illicium*.

Species.	Car-pels.	Taste.
<i>Illicium anisatum</i> .....	8	Of anise.
<i>Illicium religiosum</i> .....	8	Slightly that of laurel leaves.
<i>Illicium parviflorum</i> .....	8	Of sassafras.
<i>Illicium floridanum</i> .....	13	Of anise.
<i>Illicium griffithii</i> .....	13	Bitter and at the same time that of the leaves of laurel and of cubeb.
<i>Illicium majus</i> .....	13	Of mace.

It may be mentioned in passing that there are other species of *Illicium*. Mr. E. D. Merrill, botanist, Bureau of Science, has recently discovered in the Philippine Islands two indigenous species, *Illicium montanum* and *Illicium philippinense*.

#### THE ACTIVE PRINCIPLE AND PHARMACOLOGICAL ACTION OF ILlicium RELIGIOSUM

The chemical isolation of the poisonous principle of *Illicium religiosum* does not appear to have been the subject of many careful investigations. Eykman claimed to have succeeded in isolating from the fruit a colorless crystalline substance to which he ascribed the toxicity of *Illicium religiosum*. He provisionally named this substance "sikimine." When administered to a young dog in a dose of 12 milligrams, it produced the following symptoms:

After ten minutes: Disquiet, strong barking, twisting of the head and of the tongue in the opened mouth, scratching of the stomach and chest with the hinder extremities, afterward crossness. After fifteen minutes: Violent cramps of the muscles of the abdomen with disposition to vomit, tetanic convulsions with simultaneous extension of the four extremities, stronger flexion of the head toward the back, convulsive yawning, formation of foam, vomiting of a brown slimy mass, starting forward, sudden turning around of the entire body, afterward strong convulsions in the extremities, then paralysis, lastly collapse and death after three hours.

Langaard has carefully studied the actions of *Illicium religiosum* on rabbits, frogs, and fishes. It has been shown by him that all parts of the plant contained poison. From the results of his experiments with the alcohol-free alcoholic extract of the roots of the plant, he drew the following conclusions:

1. The poison produces convulsions by stimulating the medulla oblongata.
2. Before the appearance of the convulsions the reflex irritability in frogs is lowered, due to the stimulation of the inhibitory center in the brain. The spinal cord is not affected in the beginning, but the convulsions in the later stage of the poisoning arise from the heightened reflex excitability of the spinal cord.
3. The respiration is accelerated through the stimulation of the respiratory center.
4. Small quantities bring about slowing of the heart beat through the stimulation of the center and termination of the



vagus nerve. The irritability of the latter is depressed toward the end.

5. Small doses kill by paralyzing the respiration, while very large doses kill through paralysis of the heart.

We have not been successful in eliciting increased reflex irritability of the spinal cord in our experiments on monkeys, dogs, and cats in the interconvulsive period. There were convulsions and shaking of the entire body, but the animals did not respond to taps on the back, pinching of the tail, and moving the limbs—tests which promptly bring about convulsions in strychninized animals. The convulsions can be readily made to break out by causing the animals to struggle, for example, by lifting them up by the skin of the back. In fact it appeared that voluntary movements frequently precipitated the convulsions.

#### TREATMENT OF POISONING BY *ILlicium RELIGIOSUM*

The rational treatment of poisoning by *Illicium religiosum* will not be possible till the chemical and pharmacological reactions of its poisonous principle are definitely established. If the poison was introduced per orem, as is usually the case, the stomach should be rapidly evacuated, either by the use of apomorphine or of the stomach tube. Apomorphine offers decided advantages over the other medicinal emetics, on account of its ready action and the facility with which it can be administered, and lastly because it will produce emesis without causing further irritation of the stomach. After the stomach is evacuated, demulcent drinks or emollients may be administered. In our opinion liquid paraffin is the most preferable. It is not absorbable and is not changed by the digestive juices; hence its soothing effect will extend to the entire length of the alimentary tract. Eykman has used chloral hydrate to overcome the convulsions; Langaard considered it a life-saving remedy against the minimal lethal doses of *Illicium religiosum*. The efficacy of safe quantities of chloral hydrate against violent spinal convulsions is doubtful, since the action of such quantities on the spinal cord is little. We have used this drug in one of our cases, but from our experiments and the studies of Langaard, we believe that the best method of controlling the convulsions is by the careful inhalation of ether followed by the administration of chloral hydrate and sodium or potassium bromide; the bromides enhance the sedative effects of chloral hydrate. In the later stage of the poisoning, when paralytic symptoms predominate, these drugs are emphatically contraindicated. The



treatment in this case is chiefly a struggle to keep up the working of the paralyzed respiratory center, the circulatory apparatus, and the organs of elimination.

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RINDERPEST IN SWINE WITH EXPERIMENTS UPON ITS  
TRANSMISSION FROM CATTLE AND CARABAOS  
TO SWINE AND VICE VERSA<sup>1</sup>

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TWO PLATES AND 10 TEXT FIGURES

INTRODUCTION

This investigation was commenced with the purpose of positively determining the rôle that pigs may play in the spread of rinderpest in the Philippine Islands.

During several years of experience in handling rinderpest in the field, Dr. Stanton Youngberg, chief veterinarian, Bureau of Agriculture, has frequently noticed, and has also received reports from veterinarians on rinderpest quarantine work in the field, that in localities where rinderpest is present pigs also develop an ailment practically simultaneous with the appearance of rinderpest in cattle and in carabaos.

There is one instance where it was practically proved that pigs were the cause of an outbreak of rinderpest among cattle, carabaos, and swine in the Philippines. In February, 1908, Doctor Youngberg was ordered to investigate an outbreak of disease on Romblon Island, which was causing serious losses among cattle and carabaos.

The disease proved to be rinderpest, and this was the first time it had made its appearance on that island. The outbreak was very virulent, and the mortality was high.

It was at first difficult to account for the introduction of the disease, as no cattle or carabaos had been imported there for a long time. However, it was ascertained that hogs had been and were being imported from Capiz, a province on Panay Island and situated some 120 kilometers south of Romblon. At that time rinderpest was prevalent in Capiz Province.

Information was obtained to the effect that hogs which had been kept in the same pens with those imported from Capiz sickened first and that many died; after this, other hogs in the village became infected, and then the carabaos and the cattle began to die. During this outbreak it was noticed that when a village was found infected with rinderpest hogs had previously died in considerable numbers.

<sup>1</sup> Reprinted from *Phil. Agr. Rev.* (1916), 9, 288.

Although these facts were reported by Doctor Youngberg to the Bureau of Agriculture, the essential point of the possibility of hogs being the prime factor in the introduction of this disease was unfortunately overlooked by the authorities in charge.

The definite connection between rinderpest in cattle and in carabaos and the ailment in pigs was overlooked, because the mortality, as a rule, was not high among the pigs; the native pig is not a very thrifty-appearing individual; and swine plague, hog cholera, kidney-worm infestation, and *Balantidium coli suis* are very prevalent throughout the Islands. It was supposed that the pigs were suffering from one or more of the above-mentioned diseases and that their unthriftiness was magnified by the fact that all animals in infected areas under quarantine were watched much more closely by the veterinarians and the livestock inspectors in charge than in rinderpest-free districts.

During 1914, while I was on vacation, hog cholera broke out among the pigs at the Bureau of Agriculture stock farm at Alabang, Rizal Province. Mr. Thomas L. Bean, assistant in the veterinary research laboratory, made the hog-cholera serum and immunized these pigs. Upon completing this work, Mr. Bean left six pigs hyperimmunized to hog cholera at the laboratory. These six pigs were exposed separately to cattle sick with rinderpest, as will be noted in experiments 43 to 48 in this paper, and each one developed symptoms similar to those caused by rinderpest in cattle.

There is but little literature upon the subject of rinderpest in swine, and this little is contradictory.

Carré and Fraimbault,<sup>(2)</sup> in the course of research work upon rinderpest in Indo-China, performed some experiments upon pigs. They demonstrated the possibility of transmitting rinderpest from pig to pig by contact, as well as by blood inoculation. They also demonstrated that it was possible to transmit rinderpest by blood inoculation from bull to pig and from pig to bull. Carré and Fraimbault also mention that M. Leblanc had found rinderpest among peccaries in 1886 and that Penning had announced the transmission of rinderpest to the wild boar.

Friedberger and Frohner<sup>(3)</sup> mention Pluning as having noticed rinderpest in swine in Sumatra and that the symptoms and lesions were the same as those found in cattle. The same authors mention Theiler as being unable to transmit rinderpest to swine by inoculating them with blood from rinderpest-sick cattle.



Hutyra and Marek<sup>(4)</sup> state that the question of rinderpest in swine is not definitely settled, undoubtedly basing their statement on the contradictory results obtained by different workers. They cite Carré and Fraimbault, Penning, and Theiler.

Jobling,<sup>(5)</sup> in his annual report for 1903, cites an experiment upon rinderpest in pigs in the Philippine Islands. In this experiment he injected a farm-bred hog with 5 cubic centimeters of virulent rinderpest blood from a bull. The hog developed typical symptoms of rinderpest. Blood was taken from it and injected into a hog and a bull. The hog that received this blood did not develop any symptoms of the disease. The bull that received the blood taken from the sick pig developed typical symptoms of rinderpest, but ran a mild course of the disease and was bled to death. Upon autopsy this animal presented lesions of rinderpest, but not so far advanced as is usually noticed.

In concluding this experiment Jobling says:

This hog had the shortest incubation period of any animal I have seen, and I would have believed it to be suffering from some other disease had cow No. 46 not developed typical rinderpest after the regular incubation period.

From this one series of experiments it would appear that while the hog may contract the disease and die, and its excreta may form a source of infection for cattle and carabaos, yet it is difficult for the sick hog to reinfect others of its kind. However, no definite conclusions could be based upon the evidence at my disposal, and the work will be continued when opportunity offers.

Jobling also mentions the possibility of wild hogs spreading the disease in the provinces.

It may be mentioned here, as will be noted in the following experiments, that hogs have as a rule a shorter incubation period and show a higher temperature during the course of the disease than do cattle and carabaos. The shortness of the incubation period may be accounted for by the facts that hogs are natural scavengers and when exposed to cattle and carabaos sick with rinderpest devour large quantities of feces from these sick animals. In this way they immediately saturate their systems with large quantities of the virus, which is always present in abundance in the discharges of animals from the time of the initial rise in temperature to the time of recovery or death.

When virulent rinderpest blood taken from pigs was used in immunization work in the provinces, Doctor Youngberg noted that the virus was activated and better reactions were as a rule obtained than when the virulent blood was taken from

cattle or carabaos. Also virulent blood from hogs mixed with virulent blood from cattle or carabaos had a tendency to activate the virus.

In a personal conversation Prof. Dr. T. Horiuchi, of the Bacteriological Institute of Formosa, informed me that he had seen pigs infected with rinderpest in that island. In his experience it was of rare occurrence that cattle became infected from pigs.

#### PIGS EXPOSED TO CATTLE SICK WITH RINDERPEST

The following ten experiments were designed to furnish information regarding the possibility of pigs contracting rinderpest through contact with cattle sick with the disease.

These exposures were conducted in two stalls, which will be designated as stall No. 1 and stall No. 2. These stalls were 10 feet long by 12 feet wide. Stall No. 1 had a cement floor, and stall No. 2 had a wooden floor. The sick cattle were tied in these stalls, and the pigs were unrestrained during the exposures.

All the animals used in these experiments were kept in quarantine for a certain length of time, which will be stated in each case. While in quarantine their temperatures were taken twice a day, morning and afternoon, and each day their general physical condition was noted.

The following abbreviations will be used in connection with the data on the experiments: D., diarrhœa; E. L., eating little; N. E., not eating; D., E. L., diarrhœa, eating little; D., N. E., diarrhœa, not eating; V. B., virulent blood.

#### EXPERIMENT 1

*Pig 185.*—Known history prior to the experiment: This animal was a native pig, 7 months old, purchased in Manila, and placed in quarantine at the laboratory March 2, 1915. On May 2, 1915, it was inoculated with 50 cubic centimeters of blood from a pig suffering from hog cholera. This blood had been passed through a Berkefeld N. filter. The animal developed a mild form of hog cholera and recovered.

May 21, 1915, pig 185 was exposed to bull 3906 in stall No. 1. This exposure was continued for three days.

History of bull 3906 during the exposure:

May 21: Fifth day after the initial rise in temperature.

May 21-23: D., N. E.

May 24: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

May 25, pig 185 developed a rise in temperature, registering, in the afternoon,  $40.1^{\circ}$  C. This was four days after the initial exposure to bull 3906.

May 26, pig 185 was transferred to corral No. 1 to accomplish experiments 11 and 23.

May 27, this animal showed an afternoon temperature of  $40.9^{\circ}$  C., which was the highest temperature registered during the course of the disease. It was bled and the blood used in experiment 29.

May 28–31, N. E.

June 1–9, E. L.

This animal gradually recovered from rinderpest, but did not become thrifty, and was killed August 7, 1915.

#### EXPERIMENT 2

*Pig. 207.*—Known history prior to the experiment: This animal was a native pig, 7 months old, purchased in Manila and kept in quarantine twenty days before being used. At no time during this period did it have a high temperature or show any symptoms of sickness.

June 17, 1915, pig 207 was exposed to bull 3908, first day of temperature, and to bull 3926, sixth day of temperature. This exposure was made in stall No. 1 and was continued for five days.

History of bull 3908 during the exposure:

June 17: First day of temperature, registering, a. m.,  $38.5^{\circ}$  C., p. m.,  $39.3^{\circ}$  C.

June 22–25: D., N. E.

July 4: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

History of bull 3926 during the exposure:

June 17: Sixth day after the initial rise in temperature, N. E.

June 18–20: D., N. E.

June 21–22: D.

This animal gradually recovered.

June 21, pig 207 developed a rise in temperature, registering, in the afternoon,  $40.1^{\circ}$  C.; this was four days after the initial exposure to bulls 3908 and 3926.

June 22, pig 207 was transferred to corral No. 1 to accomplish experiment 24.

June 24, this animal showed an afternoon temperature of  $41.2^{\circ}$  C., which was the highest temperature it developed during the course of the disease.

June 30 to July 2, this animal had a slight diarrhoea and ate but little.



The pig gradually recovered from rinderpest, but did not become thrifty, and was killed on August 7, 1915.

## EXPERIMENT 3

*Pig 208.*—Known history prior to experiment: This animal was a native pig, 6 months old, purchased in Manila and kept in quarantine forty days before it was used. At no time during this period did it have a high temperature or show any symptoms of sickness.

July 19, 1915, pig 208 was exposed to bull 3939, fifth day of temperature, and to bull 3931, fourth day of temperature, in stall No. 1. This exposure was continued two days with bull 3939 and four days with bull 3931.

History of bull 3939 during the exposure:

July 19: Fifth day of temperature, D., N. E.

July 20: D., N. E.

July 21: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

History of bull 3931 during the exposure:

July 19: Fourth day of temperature.

July 21–22: N. E.

July 22: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

July 23, pig 208 developed a rise in temperature, registering, in the afternoon, 40.4° C.; this was four days after the initial exposure to bulls 3939 and 3931.

July 27, pig 208 was removed to corral No. 1 to accomplish experiments 12 and 25. It presented a morning temperature of 39.9° C. and an afternoon temperature of 41.2° C.

July 31 to August 7, E. L.

August 3, it presented an afternoon temperature of 41.6° C., which was the highest temperature registered during the course of the disease.

August 8–11, D., E. L.

August 12, died of rinderpest, presenting typical lesions of that disease upon autopsy.

## EXPERIMENT 4

*Pig 215.*—Known history prior to experiment: This animal was a native pig, 8 months old, purchased in Manila and kept in quarantine seventy-two days before it was used. At no time during this period did it have a high temperature or show any symptoms of sickness.

September 30, 1915, pig 215 was exposed to bull 3925, which was the third day after the bull's initial temperature was



recorded. This exposure was continued for four days in stall No. 1.

History of bull 3925 during the exposure:

September 30: Third day of temperature.

October 1-3: D., N. E.

October 3: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

October 3, pig 215 developed a rise in temperature, registering, in the afternoon, 40.2° C.; this was three days after the initial exposure to bull 3925.

October 4, it presented an afternoon temperature of 40.9° C., which was the highest temperature registered during the course of the disease.

October 6-12, D., N. E.

October 13-16, D.

This animal gradually recovered from rinderpest, but did not become thrifty, and was killed December 4, 1915.

#### EXPERIMENT 5

*Pig 213.*—Known history prior to experiment: This animal was a native pig, 7 months old, purchased in Manila and kept in quarantine twenty-eight days before it was used. At no time during this period did it have a high temperature or show any symptoms of sickness.

August 17, 1915, pig 213 was exposed to bull 3928, first day of temperature, in stall No. 1.

History of bull 3928 during the exposure:

August 17: First day of temperature, p. m., 39.9° C.

August 18: Temperature, a. m., 39.6° C.; p. m., 40.8° C.

August 23: N. E.

August 24-29: D., N. E.

August 30-31: E. L.

This animal gradually recovered.

August 25, pig 213 presented a rise in temperature, registering, in the afternoon, 39.9° C.; this was eight days after the initial exposure to bull 3928.

This animal showed a rather high temperature until September 10, when its temperature subsided to normal and recovery was prompt. At no time during this period did the animal present any severe symptoms, such as diarrhœa or inappetence. The disease ran a mild course throughout, which is occasionally observed in cattle and carabaos.

#### EXPERIMENT 6

*Pig 220.*—Known history prior to experiment: This animal was a native pig, 7 months old, purchased in Manila and kept

in quarantine forty-nine days before it was used. At no time during this period did it have a high temperature or show any symptoms of sickness.

October 9, 1915, pig 220 was exposed to bull 3938, second day of temperature, in stall No. 1. This exposure was continued for six days.

October 14, bull 3958, first day of temperature, was admitted to stall No. 1. This exposure was continued for eight days.

October 19, bull 3961, first day of temperature, was admitted to stall No. 1. This exposure was continued for three days.

History of bull 3938 during the exposure:

October 9: Second day of temperature, a. m., 39.9° C.

October 12-14: D., N. E.

October 15: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

History of bull 3958 during the exposure:

October 14: Initial rise in temperature, a. m., 39.3° C.; p. m., 40.4° C.

This animal ran a high temperature until October 22, when the temperature subsided to normal. It did not develop diarrhœa or inappetence, but ran a mild course of the disease.

History of bull 3961 during the exposure:

October 19: Initial rise in temperature, a. m., 39.6° C.; p. m., 40.6° C.

October 21: D.

October 22: D., N. E.

October 23: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

October 16, pig 220 showed a rise in temperature, registering, in the afternoon, 40.8° C.; this was seven days from the time of the initial exposure.

October 18 and 19, the afternoon temperatures were 40.8° and 40.9° C., respectively.

October 25, this animal's temperature had dropped to normal and recovery was prompt.

At no time did this animal present severe symptoms, such as diarrhœa and inappetence, but ran a mild course of the disease.

EXPERIMENT 7

*Pig 240.*—Known history prior to experiment: This animal was a native pig, 8 months old, purchased in Manila and kept in quarantine thirty-six days before it was used. At no time during this period did it have a high temperature or show any symptoms of sickness.

November 20, 1915, pig 240 was exposed for one day in stall No. 1 to bull 3972, which was on the sixth day after its initial rise in temperature.

Also to bull 3988 for three days in stall No. 1, this animal developing its initial rise in temperature on this day.

History of bull 3972 during the exposure:

November 20: Sixth day of temperature, D., N. E.

November 21: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

History of bull 3988 during the exposure:

November 20: Initial rise in temperature recorded; p. m. temperature, 41° C.

November 25: This animal was bled to death to secure virulent blood for immunizing purposes.

November 23, pig 240 presented a rise in temperature, registering, in the afternoon, 40.2° C.; this was three days from the time of the initial exposure.

November 24–26, N. E.

November 26, transferred to stall No. 2 to be used in experiment 27.

November 27–28, D., N. E.

November 29, E. L.

This animal gradually recovered, but later contracted hog cholera.

#### EXPERIMENT 8

*Pig 155.*—Known history prior to exposure: This animal was a Berkshire sow about 10 months old; it was brought to the laboratory from the Bureau of Agriculture experiment station at Alabang on October 13, 1915, and was kept in quarantine fifty-two days before being used. During this period at no time did it have a high temperature or show any symptoms of sickness.

December 3, 1915, pig 155 was exposed for two days to bull 3981 in stall No. 2. At the beginning of the exposure this bull was on its sixth day of temperature.

Also to bull 3983 for three days in stall No. 2. At the beginning of exposure this bull was on the fifth day of temperature.

History of bull 3981 during the exposure:

December 3: Sixth day of temperature, D., N. E.

December 4: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

History of bull 3983 during the exposure:

December 3: Fifth day of temperature, D., N. E.

December 4: D., N. E.

December 5: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

December 12, pig 155 developed a morning temperature of 40.2° C.; this was nine days from the time of the initial exposure.

This animal had a high temperature until December 21, at which date the temperature subsided to normal.

This animal ran a mild course of the disease, never showing severe symptoms such as diarrhœa and inappetence.

#### EXPERIMENT 9

*Pig 237.*—Known history prior to exposure: This animal was a native pig, 5 months old, purchased in Manila and kept in quarantine seventy-five days before it was used. During this period at no time did it have a high temperature or show any symptoms of sickness.

December 29, 1915, pig 237 was exposed to bull 3966, which was on its fifth day of temperature. This exposure lasted two days in stall No. 2.

Also to bull 3976 for five days in stall No. 2, commencing on the initial rise of temperature in the bull.

History of bull 3966 during the exposure:

December 29: Fifth day of temperature, D., N. E.

December 30: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

History of bull 3976 during the exposure:

December 29: First day of temperature, a. m., 39° C.

January 3, 1916: Bled to death for virulent blood to be used in immunization work.

January 2, 1916, pig 237 developed a morning temperature of 41.2° C.; this was four days after the initial exposure.

January 3, this pig was bled to death for virulent blood to be used in immunization work.

#### EXPERIMENT 10

*Pig 242.*—Known history prior to exposure: This animal was a native pig, 6 months old, purchased in Manila and kept in quarantine seventy-six days before it was used.

This animal arrived at the laboratory on October 15, 1915. On October 26 this pig had a high temperature, and on October 28 it developed a diarrhœa. The diarrhœa continued until November 9. The high temperature continued until November 16, when it subsided to, and remained, normal. This condition was diagnosed as hog cholera, as another pig, which had been kept in quarantine with this one, developed similar symptoms just prior to this time. It was killed, and at autopsy it presented good lesions of hog cholera.



January 30, 1916, pig 242 was exposed in stall No. 2 for three days to bull 3960, which was on its fourth day of temperature.

History of bull 3960 during the exposure:

January 30: Fourth day of temperature.

January 31: D., N. E.

February 1: Died of rinderpest, representing typical lesions of that disease.

February 2, 1916, pig 242 developed a morning temperature of  $39.8^{\circ}$  C., and an afternoon temperature of  $41^{\circ}$  C.; this was three days after the initial exposure.

February 4-5, E. L.

February 6-7, N. E.

February 7, died of rinderpest, presenting typical lesions of that disease upon autopsy.

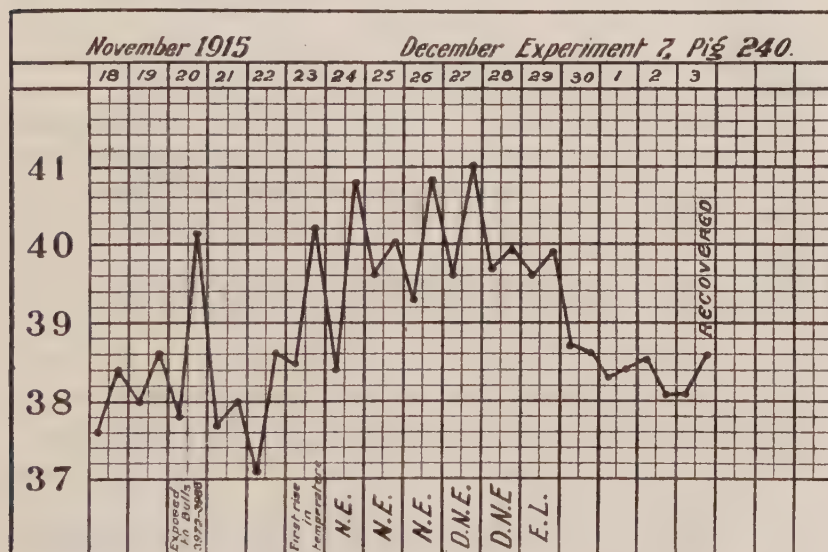


FIG. 1. Temperature chart of pig 240 exposed to bull.

In summing up the results of the ten preceding experiments on the exposure of pigs to cattle sick with rinderpest, it will be noted that all the pigs contracted a disease and developed symptoms characteristic of rinderpest in cattle and carabaos.

Three of the animals developed their initial rise in temperature on the third day, four on the fourth day, one on the seventh day, one on the eighth day, and one on the ninth day of exposure. From this it will be noted that the incubation period of rinderpest in pigs that have been exposed to cattle sick with that disease corresponds very closely to the incubation period in cattle and

carabaos, in which it varies between three and ten days, averaging five days.

Of the ten pigs used, two, or 20 per cent, died of rinderpest. Four, or 40 per cent, recovered from the disease, but were unthrifty and were killed, although under normal conditions these animals would undoubtedly have been allowed to live. Of these four, one contracted hog cholera. Three, or 30 per cent, presented very mild symptoms, showing only a temperature reaction, and recovered promptly; these animals under normal field conditions would hardly have been suspected of being sick. One, or 10 per cent, was bled to death for virulent blood, leaving the final outcome as to the effect of the disease uncertain.

These 10 pigs were exposed to 19 head of cattle sick with rinderpest. Of these 19 sick cattle, 14, or approximately 73.7 per cent, died of rinderpest; three, or approximately 15.8 per cent, recovered; two, or approximately 10.5 per cent, were bled to death for virulent blood, leaving the final outcome as to the effects of the disease uncertain.

In comparing these figures, it will be noted that the mortality in cattle was 73.7 per cent against 20 per cent in pigs and that the recovery in cattle was 15.8 per cent against 70 per cent in pigs when the mild cases and those that recovered but were unthrifty are figured together.

Thus it will be seen that rinderpest in pigs, when contracted by exposure, presents a low mortality as compared with its effect upon cattle.

#### PIGS EXPOSED TO PIGS SICK WITH RINDERPEST

The following experiments were designed to furnish information regarding the possibility of pigs contracting rinderpest by contact with pigs sick with that disease.

These exposures were conducted in two different corrals which will be designated as corral No. 1 and corral No. 2. The dimensions of corral No. 1 were 14 by 30 feet, and of corral No. 2, 16 by 30 feet. Both corrals were built on the ground, and each contained a small lean-to shed to protect the animals from the weather.

The sick pigs and those placed in the corrals to be exposed were allowed the freedom of the place.

All the animals used in these experiments were kept in quarantine a certain length of time, which will be stated in each case. While in quarantine their temperatures were taken twice a day, morning and afternoon, and each day their general physical condition was noted.

## EXPERIMENT 11

*Pig 186.*—Known history prior to the exposure: This animal was a native pig, 6 months old, purchased in Manila and brought to the laboratory and placed in quarantine on March 2, 1915.

On May 2, 1915, this animal was inoculated with 50 cubic centimeters of blood from a pig suffering from hog cholera. This blood had been passed through a Berkefeld N. filter. The animal developed a mild form of hog cholera and recovered. At no other time than when suffering from the mild attack of hog cholera did the animal have a rise in temperature or show any symptoms of sickness.

May 26, 1915, pig 186 was exposed to pig 185 in corral No. 1; this was the second day of temperature for pig 185. The exposure was continued for sixteen days.

History of pig 185 during the exposure:

Referring to experiment 1, it will be noted that pig 185 contracted the disease from bull 3906.

May 26: Temperature, a. m., 39.1° C.; p. m., 40.5° C.

May 27: Temperature, a. m., 40.2° C.; p. m., 40.9° C.

May 28-31: N. E.

June 1-9: E. L.

May 30, pig 186 developed a rise in temperature, registering, in the morning, 39.8° C.; in the afternoon, 40.6° C. This was four days after the initial exposure to pig 185.

May 31, afternoon temperature, 41.6° C., which was the highest temperature registered during the course of the disease.

June 1-4, D., N. E.

June 9-10, D., E. L.

June 15-16, D.

This animal gradually recovered, but did not become thrifty, and was killed August 7, 1915.

## EXPERIMENT 12

*Pig 212.*—Known history prior to experiment: This animal was a native pig, 5 months old, purchased in Manila and kept in quarantine eight days before it was used. On the first day in quarantine it registered an afternoon temperature of 40.4° C., which was undoubtedly due to excitement, since the temperature subsided to normal on the following day and remained so until after it was used, and at no time prior to the exposure did it manifest any symptoms of sickness.

July 27, 1915, pig 212 was exposed to pig 208 in corral No.



1; this was the fifth day of temperature for pig 208. The exposure was continued for fifteen days.

History of pig 208 before and during the exposure:

Referring to experiment 3, it will be noted that pig 208 contracted the disease from bull 3939 and bull 3931.

July 27, fifth day of temperature, a. m., 39.9° C., p. m., 41.2° C.

July 31–August 7: E. L.

August 8–11: D., E. L.

August 12: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

August 1, pig 212 developed a rise in temperature, registering, in the morning, 40.6° C.; in the afternoon, 41.6° C. This was on the fifth day after the initial exposure to pig 208.

August 3–7, E. L.

August 9–11, D., N. E.

August 11, died of rinderpest, presenting typical lesions of that disease upon autopsy.

#### EXPERIMENT 13

*Pig 218.*—Known history prior to the experiment: This animal was a native pig, 8 months old, purchased in Manila and placed in quarantine at the laboratory on August 21, 1915, where it was kept forty-five days before it was used. At no time during this period did it have a high temperature or show any symptoms of disease.

October 5, 1915, pig 218 was exposed to pig 216 in corral No. 1; this was the third day of temperature for pig 216. The exposure was continued for one day.

History of pig 216 before and during the exposure:

Referring to experiment 17 it will be noted that pig 216 was inoculated with 6 cubic centimeters of virulent rinderpest blood from bull 3925.

October 5: D., N. E., and vomiting.

October 6: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

October 9, pig 218 developed a rise in temperature, registering, in the morning, 40.4° C.; in the afternoon, 41.6° C.; this was on the fourth day after the initial exposure to pig 216.

October 11–12, E. L.

October 13, died of rinderpest, presenting typical lesions of that disease upon autopsy.

In summing up the results of experiments 11, 12, and 13, on the exposure of susceptible pigs to pigs sick with a disease they had contracted from cattle sick with rinderpest, these pigs manifesting symptoms agreeing with the symptoms of rinderpest in cattle and carabaos, and finally upon autopsy after death presenting



lesions characteristic of that disease in cattle, it will be noted that the pigs exposed to these sick ones promptly developed a disease which possessed all the characteristics of rinderpest. Two of the animals developed a rise in temperature on the fourth day after the initial exposure and one on the fifth day, which time corresponds very closely with the incubation period of rinderpest in cattle and carabaos.

Of the three animals used, two, or  $66\frac{2}{3}$  per cent, died, and one, or  $33\frac{1}{3}$  per cent, recovered, which also agrees very closely with the effects of rinderpest on cattle and carabaos.

Although the animal in experiment 11 was finally destroyed

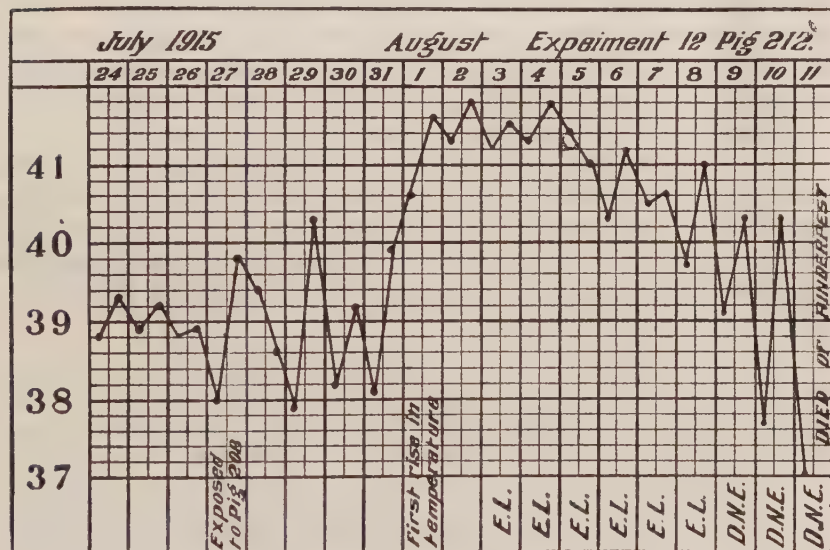


FIG. 2. Temperature chart of pig 212 exposed to pig.

on account of being unthrifty, under normal conditions in the Philippines, this animal undoubtedly would have been allowed to live.

In comparing the percentage of mortality in pigs exposed to pigs sick with rinderpest with that of pigs exposed to cattle sick with rinderpest, it appears that the virulence of the disease is increased for pigs by being passed through a pig.

#### RINDERPEST TRANSMITTED FROM PIG TO PIG BY MEANS OF THE CARETAKER

##### EXPERIMENT 14

*Pig 214.*—Known history prior to the experiment: This animal was a native pig, 8 months old, purchased in Manila and

kept in quarantine thirty-one days before it was used. At no time during this period did it have a high temperature or show any symptoms of sickness.

August 21, 1915, pig 214 was placed in corral No. 1. This experiment was designed to determine the following: (1) Whether the virus of rinderpest was still alive in such a form as to give the disease to a susceptible pig when placed in the corral, since a pig ten days previously had died of rinderpest in this corral (this was in line with other experiments that have been performed at the laboratory on cattle and carabao, (6) in which it was proved that the virus of rinderpest was in such a condition that it was unable to cause the disease after a corral had been freed from sick animals for twenty-four hours); and (2) whether the virus of hog cholera was present in the corral, since a pig, which eleven days previously had died in this corral, showed simultaneous lesions of hog cholera and of rinderpest.

Before the completion of the experiment on pig 214 in this corral, added evidence was obtained on two other points: (1) The possibility of a pig contracting rinderpest after surviving hog cholera; and (2) the possibility of transmitting, by means of the caretaker, the virus of rinderpest from a pig sick with that disease to a susceptible pig.

History of corral No. 1 immediately previous to placing pig 214 therein:

On August 11, which was eleven days previous to the beginning of this experiment, pig 212 (experiment 12) had died of rinderpest in this corral; this animal also showed lesions of hog cholera.

On August 12, which was ten days previous to the beginning of this experiment, pig 208 (experiment 3) had died of rinderpest in this corral; this animal also showed lesions of hog cholera.

August 25, 1915, pig 214 showed a rise in temperature. Its afternoon temperature remained high, ranging between 40° and 40.8° C., until September 15, when it gradually subsided to normal. During this time, although the animal did not develop diarrhoea or show marked inappetence, it was unthrifty and had a mucopurulent discharge from its eyes, presenting symptoms of a mild attack of hog cholera.

October 5, 1915, pig 216 (experiment 26) was placed in corral No. 2.

This corral was approximately 45 meters from corral No. 1, in which pig 214 was kept at that time. Pig 216 was on its second day of temperature, D., N. E. on this date, and it died of rinderpest on October 6.

Pig 218 (experiment 13) developed its first rise of temperature on October 9 and died of rinderpest four days later. These two animals were cared for by the same man that took care of pig 214 in corral No. 1.

October 14, 1915, pig 214 developed an afternoon temperature of 40.5° C. This was nine days after pig 216 was placed in corral No. 2 and eight days after its death, and five days after the initial rise in temperature of pig 218, which was also kept in corral No. 2.

October 16-18, N. E.

October 18, died of rinderpest, showing typical lesions of that disease upon autopsy.

In summing up the results of experiment 14, it will be noted that pig 214 did not contract rinderpest in corral No. 1 which had contained a pig that died of rinderpest ten days previous to the admission of pig 214 to the corral. It will also be noted that pig 214 did have a temperature and showed mild symptoms of hog cholera, which it possibly contracted in corral No. 1. It will be noted also that pig 214 did finally contract rinderpest and died of that disease and that the disease was undoubtedly brought to it by means of the caretaker.

Also it will be noted that a pig can suffer from an apparently mild attack of hog cholera and recover from that disease and later contract rinderpest and die.

#### PIGS INOCULATED WITH VIRULENT BLOOD FROM PIGS SICK WITH RINDERPEST

The following experiments were designed to furnish information regarding the possibility of pigs contracting rinderpest by inoculating them with blood taken from pigs sick with rinderpest. The animals that were inoculated were kept in screened stalls in a shed, which was free from disease, until they developed a rise in temperature, at which time they were removed.

#### EXPERIMENT 15

*Pig 206.*—Known history prior to the experiment: This animal was a native pig, 6 months old, purchased in Manila on May 28, 1915, and placed in quarantine.

May 29, 1915, pig 206 was inoculated with 10 cubic centimeters of virulent blood from pig 185.

History of pig 185 at the time the virulent blood was drawn from it:

May 29: Fourth day of temperature, N. E. The a. m. temperature was 39.4° C.; p. m. temperature, 40.1° C. For complete history see experiment 1.

June 2, pig 206 developed a rise in temperature, registering, in the afternoon, 40.2° C.; this was four days after inoculation.

June 3, this animal died. Autopsy did not reveal any marked lesions.



## EXPERIMENT 16

*Pig 219.*—Known history prior to the experiment: This animal was a native pig, 9 months old, purchased in Manila and kept in quarantine forty-five days before it was used, and at no time during this period did it have a high temperature or show any symptoms of sickness.

October 5, 1915, pig 219 was inoculated with 2 cubic centimeters of virulent blood from pig 215.

History of pig 215 at the time the virulent blood was drawn from it:

October 5: Second day after the initial rise in temperature, a. m. temperature,  $39.1^{\circ}\text{C}$ .; p. m. temperature,  $40.7^{\circ}\text{C}$ . For complete history see experiment 4.

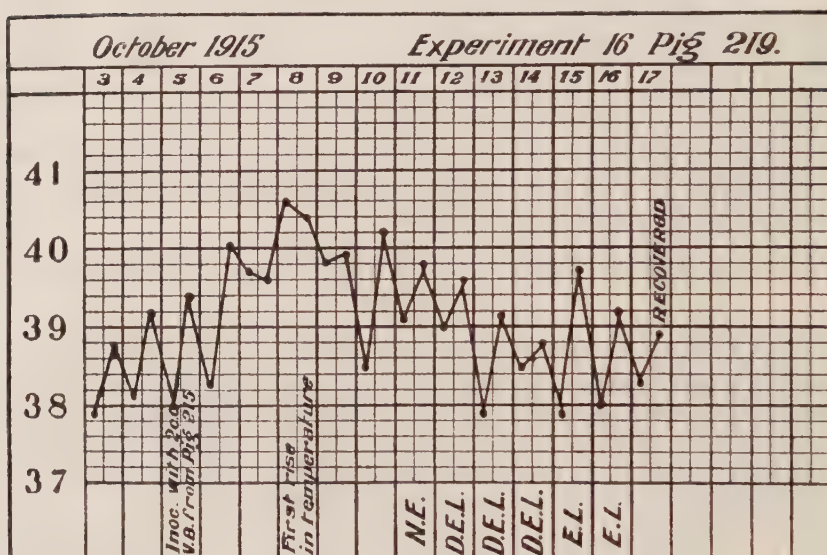


FIG. 3. Temperature chart of pig 219 inoculated with blood from pig.

October 8, pig 219 presented a rise in temperature registering, in the morning,  $40.6^{\circ}\text{C}$ .; in the afternoon,  $40.4^{\circ}\text{C}$ .; this was three days after the inoculation.

October 11, N. E.

October 12-14, D., N. E.

October 15-16, E. L.

This animal gradually recovered, but did not become thrifty and was killed December 4, 1915.

In summing up the results of experiments 15 and 16, it can not be stated with certainty that pig 206 used in experiment 15 contracted rinderpest, on account of its early death and the absence of the lesions that are to be expected in the early stage



of the disease and which have been found in cattle and carabaos under similar conditions. However, the animal had an incubation period that corresponded to that of rinderpest.

In experiment 16, pig 219 ran a typical course of rinderpest and recovered with the after effects of being unthrifty, which condition is frequently noticed in pigs after they have recovered from rinderpest.

#### PIGS INOCULATED WITH VIRULENT BLOOD FROM CATTLE SICK WITH RINDERPEST

The following experiments were designed to furnish information regarding the possibility of pigs contracting rinderpest by inoculating them with blood taken from cattle sick with rinderpest. The animals that were inoculated were kept in screened stalls in a shed, which was free from disease, until they developed a rise in temperature, at which time they were removed.

#### EXPERIMENT 17

*Pig 216.*—Known history prior to the experiment: This animal was a native pig, 7 months old, purchased in Manila and kept in quarantine seventy-two days before it was used. At no time during this period did it develop a temperature or show any symptoms of disease.

September 30, 1915, pig 216 was inoculated with 6 cubic centimeters of virulent blood from bull 3925, which was on its second day of temperature.

##### History of bull 3925:

September 25: Inoculated with 200 cubic centimeters of virulent blood from bull 3937.

September 28: First rise in temperature, p. m., 40.4° C.

September 30: Second day of temperature, blood was taken to inoculate pig 216.

October 1-3: D., N. E.

October 3: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

October 3, pig 216 developed a rise in temperature, registering, in the morning, 40.5° C.; in the afternoon, 41.1° C.; this was three days after the inoculation.

October 5, D., N. E., and vomiting.

October 6, died of rinderpest, presenting marked lesions of that disease upon autopsy. This pig was used in exposure experiments 13 and 26.

## EXPERIMENT 18

*Pig 241.*—Known history prior to the experiment: This animal was a native pig 6 months old, purchased in Manila and kept in quarantine one hundred fifteen days before it was used. It developed a high temperature on October 26, 1915, and was taken out of the quarantine shed and isolated. It developed a diarrhoea October 28, which lasted until November 7, when the diarrhoea subsided, and the temperature gradually returned to normal. During this period the animal presented a clinical picture of a mild case of hog cholera. November 26 pig 241 was returned to the quarantine shed, where it remained until it was used, and did not show a rise of temperature or any symptoms of sickness.

February 7, 1916, pig 241 was inoculated with 25 cubic centimeters of virulent rinderpest blood drawn from bull 3962, which was on its third day of temperature. Pig 241 was placed in a screened stall in a shed free from rinderpest.

## History of bull 3962:

February 1, 1916: Bull 3962 was inoculated with 100 cubic centimeters of virulent rinderpest blood from bull 3960.

February 4: Bull 3962 developed a rise in temperature, registering, a. m., 39.8° C.; p. m., 40.6° C.

February 5-8: D., N. E.

February 8: This animal presented a subnormal a. m. temperature of 35.8° C., and died, presenting typical lesions of rinderpest upon autopsy.

On the day the blood was drawn to inoculate into pig 241, this bull had diarrhoea and was not eating.

February 10, pig 241 developed a rise in temperature, registering, in the morning, 40° C.; in the afternoon, 41.3° C.; this was the third day after inoculation.

February 13, N. E.

Pig 241 died during the night of February 13 and presented typical lesions of rinderpest upon autopsy.

## EXPERIMENT 19

*Pig 239.*—Known history prior to the experiment: This animal was a native pig, 8 months old, purchased in Manila and kept under observation one hundred twenty-two days before it was used. It developed a high temperature October 27, 1915, and was taken out of the quarantine shed and isolated. It developed a diarrhoea October 28, which continued until November 7, at which time the diarrhoea subsided,

and the temperature returned to normal. During this period the animal presented a clinical picture of suffering from a mild attack of hog cholera. November 26 pig 239 was returned to the quarantine shed, where it remained until it was used, and did not develop a rise in temperature or show any symptoms of sickness during this period.

February 14, 1916, pig 239 was inoculated with 20 cubic centimeters of blood from bull 3993, which had been bled to death February 13 for virulent blood to be used in immunization work. The blood that was inoculated into the pig had been kept twenty-four hours in the ice box.

History of bull 3993 from which the blood was taken:

February 5, 1916: Drenched with 100 cubic centimeters of urine from pig 242 (experiment 35).

February 9: First rise of temperature, registering, p. m., 40.5° C.

February 12-13: E. L.

February 13: Bled to death for virulent blood to be used in immunization work.

Pig 239 did not develop a rise in temperature or show any symptoms of sickness from this injection and afterward was exposed in shed No. 3, where the animals sick with rinderpest were kept, and continued to give the same negative results.

#### EXPERIMENT 20

*Pig 154.*—Known history prior to the experiment: This animal was a Berkshire, about 1 year old; it was brought to the laboratory from the Bureau of Agriculture experiment station at Alabang on October 13, 1915, and was kept in quarantine at the laboratory one hundred thirty-two days before it was used. During this period the animal did not show a rise in temperature. January 10-11, 1916, it had diarrhœa, which was undoubtedly due to dietetic conditions.

February 23, 1916, pig 154 was inoculated with 8 cubic centimeters of blood from cow 3995, which had been bled to death on February 21 for virulent blood to be used in immunization work. The blood that was inoculated into pig 154 had been kept forty-eight hours in the ice box.

History of cow 3995 from which the blood was taken:

February 11, 1916: This animal was exposed to pig 241 (experiment 28).

February 17: First rise in temperature, registering, p. m., 40.2° C.

February 20: D., E. L.

February 21: D., N. E. On this date it was bled to death for virulent blood to be used in immunization work.



Pig 154 did not develop a rise in temperature or show any symptoms of sickness from this injection. It was later exposed in shed No. 3, where animals sick with rinderpest were kept, and it continued to give the same negative results.

In summing up the results of experiments 17, 18, 19, and 20, it will be noted that the pigs used in experiments 17 and 18 had an incubation period of three days and ran a severe course of the disease and died, presenting typical lesions of rinderpest upon autopsy.

Both of the pigs used in experiments 19 and 20 ran a high

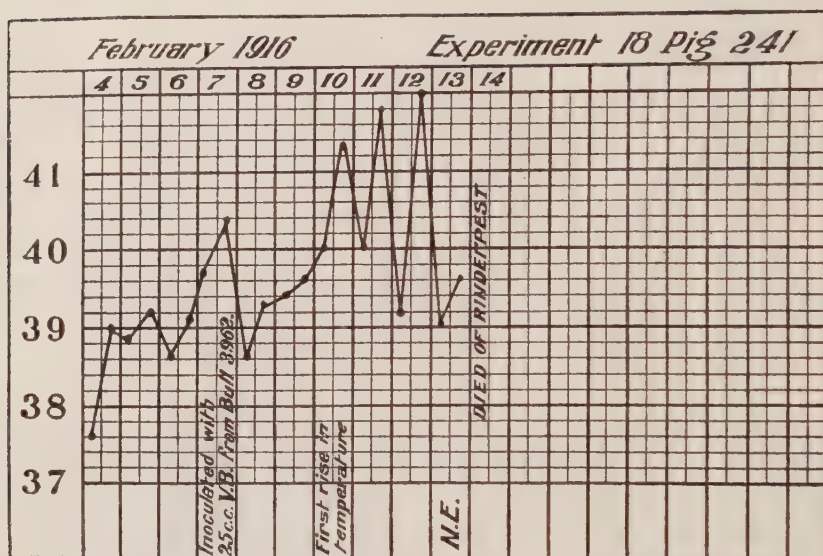


FIG. 4. Temperature chart of pig 241 inoculated with blood from bull.

temperature and developed diarrhoea previous to being used in these experiments. At the time they were sick, it was thought that they were suffering from hog cholera; however, it is possible that they were suffering from a mild form of rinderpest, as it is almost impossible to distinguish the two diseases in pigs, unless autopsy is made or their blood is inoculated into susceptible cattle. These animals did not contract rinderpest when inoculated with virulent blood from cattle, and they did not contract rinderpest by subsequent exposures. The blood with which they were injected was proved to be virulent, as in both cases it was used in immunizing work with good results.

Hence pigs 239 and 154 were either naturally immune to rinderpest or they had suffered from a previous attack of rinderpest and had acquired immunity.



PIGS INOCULATED WITH VIRULENT BLOOD FROM CATTLE AND PIGS  
SICK WITH RINDERPEST

The following experiment was designed to furnish information regarding the possibility of pigs contracting rinderpest when inoculated with a mixture of virulent rinderpest blood from cattle and pigs.

EXPERIMENT 21

*Pig 244.*—Known history prior to the experiment: This animal was a native pig, 6 months old, purchased in Manila and kept in quarantine seventy-eight days before it was used. At no time during this period did it have a high temperature or show any symptoms of sickness.

January 4, 1916, pig 244 was inoculated with a mixture of 25 cubic centimeters of virulent rinderpest blood from bull 3976 and pig 237.

History of bull 3976 from which blood was taken:

December 28, 1915: Bull 3976 was inoculated with 100 cubic centimeters of virulent rinderpest blood procured from a sick carabao in Pampanga Province.

December 31: First rise in temperature, registering, a. m., 39.1° C.

January 3, 1916: Bled to death for virulent blood to be used in immunizing work.

For history of pig 237, from which blood was taken, see experiment 9.

January 7, pig 244 developed a rise in temperature, registering, in the morning, 39.8° C.; in the afternoon, 40.6° C.; this was three days after the inoculation.

January 10, this animal's afternoon temperature was 41.3° C., which was the highest temperature registered during the course of the disease.

January 10-24, D., E. L.

January 24, died of rinderpest, presenting good lesions of that disease upon autopsy.

In summing up the results of this experiment it will be noted that a pig will contract rinderpest when inoculated with a mixture of virulent rinderpest blood from cattle and pigs.

PIGS DRENCHED WITH VIRULENT BLOOD FROM CARABAOS SICK WITH  
RINDERPEST

The following experiment was designed to furnish information regarding the possibility of pigs contracting rinderpest when given virulent rinderpest blood from carabaos by drench.

## EXPERIMENT 22

*Pig 243.*—Known history prior to the experiment: This animal was a native pig, 6 months old, purchased in Manila and kept in quarantine ninety-two days before it was used. At no time during this period did it register a high temperature. On January 10 and 11 it had diarrhoea, which immediately subsided and was undoubtedly due to dietetic conditions.

January 18, 1916, pig 243 was drenched with 50 cubic centimeters of virulent rinderpest blood, procured from a carabao sick with rinderpest from Pampanga Province.

January 22, pig 243 presented a rise in temperature, registering, in the afternoon, 40.2° C.; this was four days after it had received the drench.

January 23, morning temperature, 41.0° C.

January 24, bled to death for virulent blood to be used in immunization work.

In summing up the results of this experiment, it will be noted that a pig will contract rinderpest when drenched with virulent rinderpest blood taken from a carabao sick with rinderpest.

## CATTLE EXPOSED TO PIGS SICK WITH RINDERPEST

The following experiments were designed to furnish information regarding the possibility of cattle contracting rinderpest when exposed to pigs sick with rinderpest.

The experiments were conducted in corrals Nos. 1 and 2 and in stall No. 1.

The cattle used in these experiments were obtained from Batan Island. As, to our knowledge, there has never been any rinderpest on this island, these cattle are highly susceptible.

## EXPERIMENT 23

*Bull 3908.*—Known history prior to the experiment: Native Batanes bull, 5 years old, received at the laboratory and placed in quarantine March 13, 1915. This animal was kept under observation seventy-four days before it was used, and at no time during this period did it present a high temperature or develop any symptoms of sickness.

May 26, 1915, bull 3908 was placed in corral No. 1 with pig 185, which was on its second day of temperature (see experiment 1), and the exposure was continued for fifteen days.

May 30, bull 3908 was also in contact with pig 186, first day of temperature (see experiment 11), and the exposure was continued for twelve days.

(For history of pigs 185 and 186 see experiments 1 and 11.)

Bull 3908 did not develop any symptoms from these exposures.

June 10, bull 3908 was inoculated with 200 cubic centimeters of virulent rinderpest blood from bull 3840 (see experiment 29).

June 17, first rise in temperature, registering, in the afternoon, 39.3° C., which was the highest temperature during the course of the disease; this was seven days after the inoculation.

June 15–20, D. It will be noted that the animal had a diarrhoea three days before its slight rise in temperature.

June 21–25, D., N. E.

June 26–July 3, D., E. L.

July 4, died of rinderpest, presenting good lesions of that disease upon autopsy.

#### EXPERIMENT 24

*Bull 3924.*—Known history prior to the experiment: Native Batanes bull, 3 years old, received at the laboratory and placed in quarantine April 13, 1915. This animal was kept under observation seventy days before it was used, and at no time during this period did it present a high temperature or show any symptoms of sickness.

June 22, 1915, bull 3924 was exposed in corral No. 1 to pig 207, which was on the second day of its temperature (see experiment 2). This exposure was continued twenty-two days.

History of pig 207 (see experiment 2):

June 22: Temperature, p. m., 40.8° C.

June 23: Temperature, p. m., 40.9° C.

June 24: Temperature, p. m., 41.2° C.

June 25: Temperature, p. m., 40.8° C.

June 30–July 2: D.

This animal gradually recovered, but never became thrifty.

Bull 3924 did not show a rise in temperature or present any symptoms of sickness during this exposure.

July 3, bull 3924 was inoculated with 100 cubic centimeters of virulent rinderpest blood taken from a carabao in Imus.

July 8, inoculated with 10 cubic centimeters of virulent rinderpest blood from bull 3930.

July 11, bull 3924 presented an afternoon temperature of 40° C. This was the highest and only rise in temperature this animal showed.

July 17, inoculated with 5 cubic centimeters of blood taken from bull 3929, which showed *Anaplasma* in its blood.

July 21–22, D.

September 13–14, D.

September 17, died, the cause of death not being ascertained.



## EXPERIMENT 25

*Bull 3935.*—Known history prior to the experiment: Native Batanes bull, 2 years old, received at the laboratory and placed in quarantine June 16, 1915. This animal was kept under observation forty-one days before it was used, and at no time during this period did it present a high temperature or show any symptoms of sickness.

July 27, 1915, bull 3935 was exposed in corral No. 1 to pig 208, which was on its fifth day of temperature. This exposure was continued for sixteen days. This bull was also exposed on August 1 to pig 212 on the first day of temperature. This exposure was continued for eleven days.

History of pig 208 (see experiment 3):

July 27: It presented an a. m. temperature of  $39.5^{\circ}$  C. and a p. m. temperature of  $41.2^{\circ}$  C.

July 31–August 7: E. L.

August 3: It presented a p. m. temperature of  $41.6^{\circ}$  C., which was the highest temperature registered during the course of the disease.

August 8–11: D., E. L.

August 12: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

History of pig 212 (see experiment 12):

August 1: Temperature, a. m.,  $40.6^{\circ}$  C.; p. m.,  $41.6^{\circ}$  C.

August 3–7: E. L.

August 9–11: D., N. E.

August 11: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

Bull 3935 did not show a rise in temperature or develop any symptoms of sickness from these two exposures.

September 16, 1915, bull 3935 was inoculated with 100 cubic centimeters of virulent rinderpest blood procured from a sick carabao in Pampanga Province.

September 20, bull 3935 presented a morning temperature of  $39.6^{\circ}$  C. and was bled to death for virulent blood to be used in immunization work.

## EXPERIMENT 26

*Bull 3886.*—Known history prior to the experiment: Native Batanes bull, 3 years old, received at the laboratory and placed in quarantine November 7, 1914. This animal was kept under observation three hundred thirty-two days before it was used in this experiment, and at no time during this period did it have a high temperature or show any symptoms of sickness.

It had been used in two previous experiments which were as follows:



February 19, 1915, inoculated with 10 cubic centimeters of virulent rinderpest blood, which had been heated at 58° C. in a water bath for one hour. The animal did not develop any reaction from this.

August 28, 1915, this animal was inoculated with a culture made from rinderpest blood. The animal did not develop any reaction from this.

October 5, 1915, bull 3886 was exposed in corral No. 2 to pig 216, which was on the second day of temperature. This exposure lasted one day. This bull was also exposed on October 9 to pig 218, which was on the first day of temperature. This exposure was continued for nine days.

This bull was also exposed by means of the caretaker on October 13 to pig 214. This exposure was continued for five days.

History of pig 216 (see experiment 17):

October 5: D., N. E., and vomiting.

October 6: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

History of pig 218 (see experiment 13):

October 9: First rise in temperature, registering, a. m., 40.4° C.; p. m., 41.6° C.

October 11-12: E. L.

October 13: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

History of pig 214 (see experiment 14):

October 14: First rise in temperature.

October 16-18: N. E.

October 18: Died of rinderpest, showing typical lesions of that disease upon autopsy.

October 20, bull 3886 developed a rise in temperature, registering, in the afternoon, 40.2° C.

October 25-26, N. E.

October 27-30, D., N. E.

October 31-November 7, D., E. L.

November 7-11, D., N. E.

November 12, died of rinderpest, presenting typical lesions of that disease upon autopsy.

#### EXPERIMENT 27

*Bull 3979.*—Known history prior to the experiment: Native Batanes bull, 1 year and 4 months old, received at the laboratory and placed in quarantine October 23, 1915. This animal was kept under observation thirty-four days before it was used, and at no time during this period did it have a high temperature or show any symptoms of sickness.

November 26, 1915, bull 3979 was exposed four days in stall No. 2 to pig 240, which was on its third day of temperature.

History of pig 240 (see experiment 7):

November 26: N. E. Temperature, p. m. 40.8° C.

November 27-28: D., N. E.

November 29: E. L.

This animal gradually recovered, but later contracted hog cholera.

Bull 3979 presented no reaction to this exposure.

December 9, 1915, bull 3979 was inoculated with 150 cubic centimeters of virulent blood from bull 3963.

December 12, first rise in temperature.

December 13-14, N. E.

December 14, bled to death for virulent blood to be used in immunization work.

#### EXPERIMENT 28

*Cow 3995.*—Known history prior to the experiment: Native Batanes cow, 13 years old, received at the laboratory and placed in quarantine January 19, 1916. This animal was kept under observation twenty-three days before it was used, and at no time during this period did it have a high temperature or show any symptoms of sickness.

February 11, 1916, cow 3995 was exposed for four days in stall No. 2 to pig 241, which at the time of the exposure was on its second day of temperature.

History of pig 241 (see experiment 18):

February 11: Temperature, a. m., 40° C.; p. m., 41.8° C.

February 12: Temperature, a. m., 39.2° C.; p. m., 42° C.

February 13: N. E.

Pig 241 died during the night of February 13 and presented typical lesions of rinderpest upon autopsy.

February 17, cow 3995 presented a rise in temperature, registering, in the afternoon, 40.2° C.

February 18, temperature, morning, 39.2° C.; afternoon, 40.7° C.

February 20, D., E. L.

February 21, D., N. E., bled to death for virulent blood to be used in immunization work.

In summing up the results obtained in experiments 23 to 28, inclusive, it will be noted that cattle can contract rinderpest from pigs by being exposed by contact in a corral or stall to pigs sick with rinderpest, although the transmission of the disease does not appear to be so constant as by other methods; the reason for this has not been ascertained.

In experiment 23 it will be noted that bull 3908 and pig 186 (experiment 11) were exposed in the same corral and at the same time to pig 185 and that pig 186 contracted the disease but the bull was unaffected. It will also be noted that bull 3840 (experiment 29) was inoculated with blood from pig 185 on May 27 and contracted rinderpest from this blood, which proves that the blood of pig 185 was virulent when bull 3908 was exposed to it. It will also be noted that bull 3908 was exposed to pig 186 through the entire course of the disease, including the incubation period, and remained unaffected. Finally it will be noted that bull 3908 was inoculated with virulent rinderpest blood which was taken from bull 3840 and that bull 3908

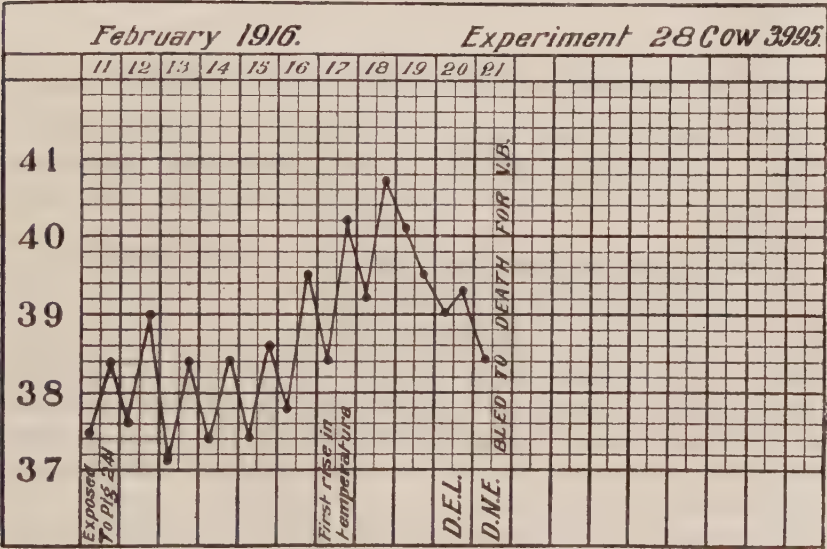


FIG. 5. Temperature chart of cow 3995 exposed to pig.

contracted rinderpest from this inoculation and died. Thus, by inoculation, bull 3908 was killed with the same virus to which it had been exposed in pig 185 without effect.

In experiment 24 bull 3924 was either naturally immune to rinderpest, a condition of rare occurrence, or it contracted a very mild type of disease from the exposure to pig 207 and thereby developed an active immunity, since it was unaffected by subsequent inoculations of virulent rinderpest blood.

In experiment 25 it will be noted that bull 3935 and pig 212 (experiment 12) were exposed in the same corral and at the same time to pig 208 and that pig 212 contracted the disease but bull 3935 remained unaffected. It will also be noted that



bull 3935 was exposed to pig 212 through the entire course of the disease, including the incubation period, and that the bull remained unaffected. However, when this animal was inoculated with virulent blood, it contracted rinderpest, which proves that it was susceptible during the exposures, but for some unknown reason it failed to contract rinderpest by contact with the pigs.

In experiment 26 bull 3886 contracted rinderpest and died of that disease by being exposed to pigs sick with rinderpest, which proves that it is possible for cattle to contract rinderpest by being exposed to pigs sick with that disease.

Experiment 28 gives proof similar to that of experiment 26, showing that it is possible for cattle to contract rinderpest from pigs by exposure.

#### CATTLE INOCULATED WITH BLOOD FROM PIGS SICK WITH RINDERPEST

The following experiments were designed to furnish information regarding the possibility of cattle contracting rinderpest when inoculated with blood from pigs which were sick with that disease.

The cattle used in these experiments were of a similar type to those used in the preceding experiments.

#### EXPERIMENT 29

*Bull 3840.*—Known history prior to the experiment: Native Batanes bull,  $4\frac{1}{2}$  years old, received at the laboratory and placed in quarantine September 28, 1914. This animal was kept under observation for two hundred forty-one days before it was used in this experiment.

On October 6, 1914, this animal was inoculated with 50 cubic centimeters of blood taken from a sick animal at Calamba. Bull 3840 did not present any reaction to this inoculation.

October 18, 19, and 20 this animal presented afternoon temperatures of  $39.9^{\circ}$ ,  $40^{\circ}$ , and  $39.9^{\circ}$  C., respectively. At no other time during this period of observation did it present a high temperature, and at no time did it show any symptoms of sickness.

May 27, 1915, bull 3840 was inoculated with 20 cubic centimeters of virulent blood from pig 185. (For history of pig 185 see experiment 1.)

May 30, bull 3840 presented a rise in temperature, registering  $40^{\circ}$  C. in the afternoon.

June 5–6, N. E.

June 7–9, D., E. L.



This animal gradually recovered, and was later used in making hyperimmune rinderpest serum.

August 19, bull 3840 received subcutaneously 1,000 cubic centimeters of virulent rinderpest blood from bull 3928. This injection caused a slight rise in temperature on the following day, but the animal suffered no other disturbance.

September 20, bull 3840 received subcutaneously 1,000 cubic centimeters of virulent rinderpest blood from bull 3935. This injection caused practically no reaction.

#### EXPERIMENT 30

*Bull 3937.*—Known history prior to the experiment: Native Batanes bull, 4 years and 9 months old, received at the laboratory and placed in quarantine June 16, 1915. This animal was kept under observation forty-eight days before it was used, and at no time during this period did it have a high temperature or show any symptoms of sickness.

August 3, 1915, bull 3937 was inoculated with 0.5 cubic centimeter of blood from pig 212.

History of pig 212 (see experiment 12):

August 3: E. L.

Bull 3937 presented no reaction to this inoculation.

September 20, bull 3937 was inoculated with 100 cubic centimeters of virulent rinderpest blood from bull 3935.

September 23, rise in temperature.

September 26-27, D., N. E.

September 27, died of rinderpest, presenting typical lesions of that disease upon autopsy.

#### EXPERIMENT 31

*Bull 3839.*—Known history prior to the experiment: Native Batanes bull, 4 years old, received at the laboratory and placed in quarantine September 28, 1914. This animal was kept under observation three hundred seventy-two days before it was used in this experiment.

October 10, 1914, this animal presented an afternoon temperature of 40° C. and was isolated from the quarantine shed.

October 11, D.

October 12, D., E. L.

October 18, this animal was returned to the quarantine shed, as its temperature and physical appearance were normal. The temperature and diarrhoea were undoubtedly due to dietary conditions.

May 4, 1915, this animal developed a diarrhoea which lasted but one day.

May 21, 22, and 23, this animal again developed a diarrhoea, but did not present a high temperature and soon returned to normal.

August 24, 1915, bull 3839 was inoculated with a culture in an experiment on the cultivation of the rinderpest virus, but the animal failed to show any reaction to this inoculation.

October 5, 1915, bull 3839 was inoculated with 3 cubic centimeters of virulent rinderpest blood from pig 216.

History of pig 216 (see experiment 17):

October 5: D., N. E., vomiting.

October 6: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

October 9, bull 3839 presented a morning temperature of 39.8° C.

October 12, 13, and 14, D., N. E.

October 15, died of rinderpest, presenting typical lesions of that disease upon autopsy.

#### EXPERIMENT 32

*Cow 3958.*—Known history prior to the experiment: Native Batanes cow, 2 years old, received at the laboratory and placed in quarantine October 1, 1915. This animal was kept under observation ten days before it was used.

October 5, D. The animal did not possess a temperature, however, and the bowels were normal the following day.

October 10, 1915, cow 3958 was inoculated with 50 cubic centimeters of virulent rinderpest blood from pig 217.

History of pig 217:

Contracted disease by exposure to sick pigs.

October 8: First rise in temperature, registering, a. m., 39.7° C.; p. m., 41° C.

October 11: D., E. L.

October 14, cow 3958 presented a morning temperature of 39.3° C.; afternoon temperature, 40.4° C.

This animal showed a high temperature until October 22, when its temperature subsided to normal. It did not develop inappetence or diarrhoea during the course of the disease and was proved immune by subsequent exposures in the shed where the animals sick with rinderpest were kept.

In summing up the results obtained in experiments 29 to 32, inclusive, it will be noted that cattle readily contract rinderpest when inoculated with blood taken from pigs sick with that disease.

In experiment 30 the bull failed to contract the disease when inoculated with 0.5 cubic centimeter of blood. Although this is a very small amount, yet such a quantity is usually sufficient to give the disease, and a much smaller quantity has been demonstrated to transfer the disease when the virulent blood was taken from a sick bull.(1)

In experiment 32 cow 3958 was inoculated with blood from pig 217 to ascertain whether the pig had contracted rinderpest or hog cholera in the quarantine shed, the result showing that the pig had contracted rinderpest.

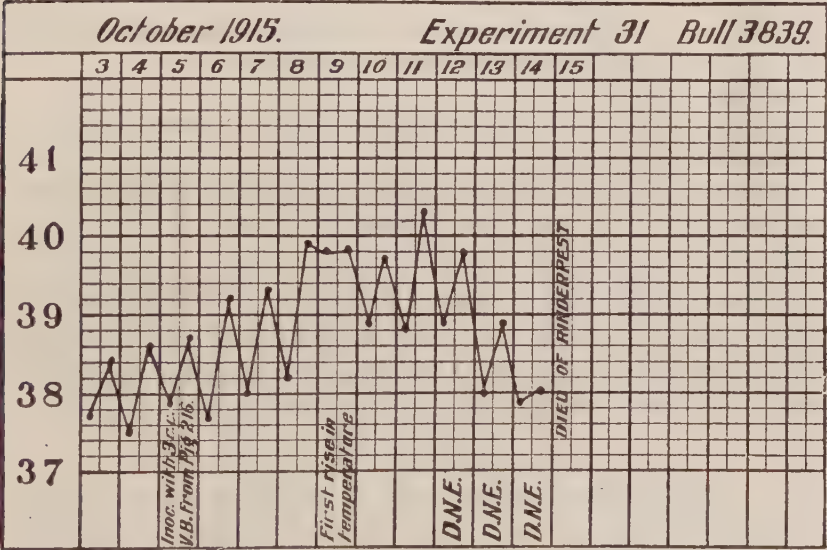


FIG. 6. Temperature chart of bull 3839 inoculated with blood from pig.

CATTLE INOCULATED WITH A MIXTURE OF BLOOD FROM PIGS AND CATTLE SICK WITH RINDERPEST

The following experiment was designed to furnish information regarding the possibility of cattle contracting rinderpest when inoculated with a mixture of blood from pigs and cattle sick with rinderpest, as it was desired in several instances to obtain more blood for immunization work than would be furnished by one bull. Thus, if the virus maintained its potency by mixing pig and cattle blood, pigs frequently could be bled to death to supply the demand.

EXPERIMENT 33

Cow 3987.—Known history prior to the experiment: Native Batanes cow, 1 year and 2 months old, received at the laboratory and placed in quarantine October 23, 1915. This animal



was kept under observation forty-three days before it was used in this experiment.

December 31, 1915, D.

January 1, 1916, D.

January 2-3, D., E. L. This condition was undoubtedly due to dietary conditions, as the animal did not present a high temperature.

January 4, cow 3987 was inoculated with 150 cubic centimeters of mixed virulent blood from pig 237 and bull 3976. (For history of pig 237, see experiment 9.) (For history of bull 3976, see experiment 21.)

January 7, cow 3987 presented a morning temperature of 39.1° C. and an afternoon temperature of 40.4° C.

January 9, E. L.

January 10, E. L., bled to death for virulent blood to be used in immunization work.

In summing up the results of this experiment it will be noted that the potency of the virulent blood is not materially affected by mixing pig and cattle blood.

#### CATTLE DRENCHED WITH URINE FROM PIGS SICK WITH RINDERPEST

The following experiments were designed to furnish information regarding the possibility of cattle contracting rinderpest from the urine of pigs suffering with rinderpest.

#### EXPERIMENT 34

*Cow 3978.*—Known history prior to the experiment: Native Batanes bull, 2 years old, received at the laboratory and placed in quarantine October 23, 1915. It was kept under observation fifty-two days before it was used. During this period it did not present a high temperature or show any symptoms of sickness.

December 14, 1915, bull 3978 was drenched with 30 cubic centimeters of urine from pig 155. Water was added to the urine, making the quantity 250 cubic centimeters. This urine had also stood overnight.

History of pig 155 (see experiment 8):

December 14: Temperature, a. m., 40.2° C.; p. m., 40.6° C.

This animal ran a mild course of the disease and did not develop inappetence or diarrhoea.

Bull 3978 did not develop any reaction to this drench.

January 18, 1915, bull 3978 was inoculated with 400 cubic centimeters of virulent rinderpest blood procured from a carabao sick with rinderpest in Pampanga Province.



January 22, bull 3978 presented a rise in temperature.

January 24, this animal was bled to death for virulent blood to be used in immunization work.

# EXPERIMENT 35

*Bull 3993.*—Known history prior to experiment: Native Batanes bull, 7 years old, received at the laboratory and placed in quarantine January 19, 1916, and kept under observation sixteen days before it was used in this experiment. At no time

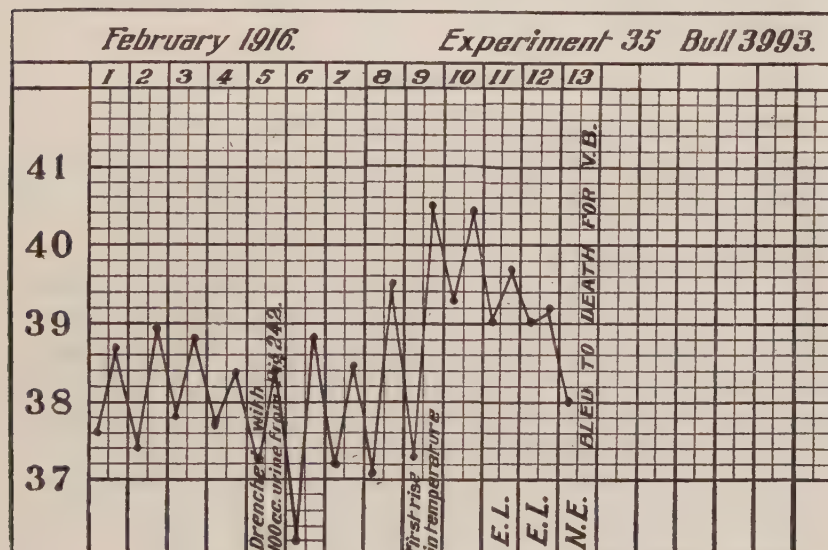


Fig. 7. Temperature chart of bull 3993 drenched with urine from pig.

during this period did it have a high temperature or show any symptoms of disease.

February 5, 1916, bull 3993 was drenched with 100 cubic centimeters of fresh undiluted urine from pig 242.

History of pig 242 (see experiment 10):

February 5: E. L.; a. m. and p. m. temperatures, 40.4° C.

February 6: N. E.

February 7: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

February 9, bull 3993 presented an afternoon temperature of 40.5° C.

February 12 and 13, E. L.

February 13, N. E., and was bled to death for virulent blood to be used in immunization work.

In summing up the results of these two experiments, with special reference to experiment 35, it will be noted that cattle

can contract rinderpest by being drenched with urine from a pig sick with rinderpest. In experiment 34 there is a possibility that the urine was kept too long before being used, as has been demonstrated in previous work.<sup>(5)</sup>

#### CARABAOS EXPOSED TO PIGS SICK WITH RINDERPEST

The following experiment was designed to furnish information regarding the possibility of carabaos contracting rinderpest by being placed in contact with pigs sick with rinderpest. This experiment was conducted in stall No. 2.

#### EXPERIMENT 36

*Carabao 57.*—Known history prior to the experiment: This animal was a native carabao from Aparri, 8 years old, received at the laboratory and placed in quarantine May 11, 1916, and kept under observation seventy-two days before it was used in this experiment.

June 17, 1916, carabao 57 was used in an experiment in which an investigation was being conducted by Dr. H. W. Wade, of the Bureau of Science, and myself for the purpose of producing a vaccine for rinderpest by attenuating the virus by means of desiccated virulent blood and tissue pulp. This investigation will be reported on at a later date.

Carabao 57 gave no reaction to the injections administered. July 22, 1916, carabao 57 was exposed to pigs 299 and 301.

##### History of pig 299:

July 17, 1916: Inoculated with 50 cubic centimeters of virulent blood from carabao 53.

July 20: First rise in temperature.

July 21-23: N. E.

July 24: E. L.

July 25-26: D., E. L.

July 27: Died of rinderpest, presenting typical lesions of that disease upon autopsy.

##### History of pig 301:

July 17, 1916: Inoculated with 50 cubic centimeters of virulent rinderpest blood from carabao 53.

July 19: First rise in temperature.

July 21-23: N. E.

July 24-27: E. L.

This animal gradually recovered.

July 29, carabao 57 presented a rise in temperature, registering 39.3° C. in the morning.

July 31, D., N. E.

August 1-2, D., N. E.

August 3, died of rinderpest, presenting typical lesions of that disease upon autopsy.

In summing up the results of this experiment, it is proved that a carabao can contract rinderpest from pigs sick with that disease when the carabao is exposed to the pigs by direct contact.

#### CARABAOS INOCULATED WITH BLOOD FROM PIGS SICK WITH RINDERPEST

The following experiment was designed to furnish information regarding the possibility of carabaos contracting rinderpest

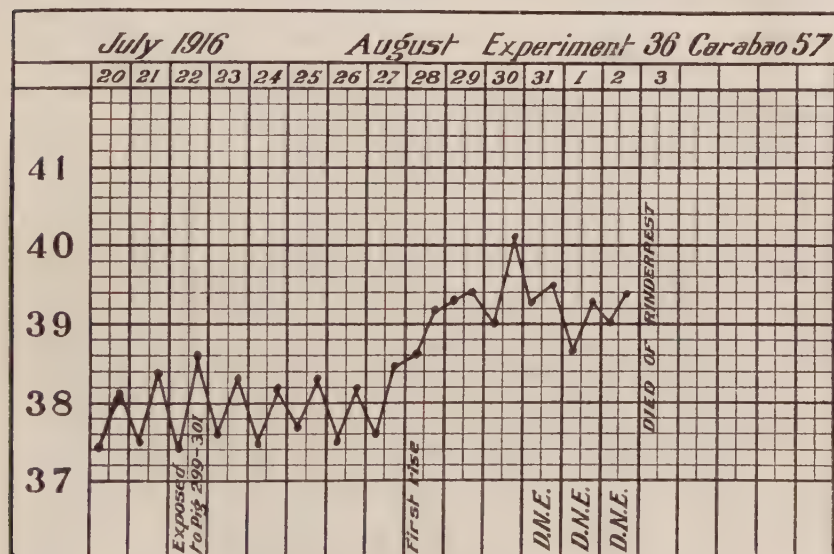


FIG. 8. Temperature chart of carabao 57 exposed to pig.

from pigs when inoculated with blood from pigs sick with rinderpest.

#### EXPERIMENT 37

*Carabao 51.*—Known history prior to the experiment: This animal was a native carabao from Aparri, 6 years old; it was received at the laboratory and placed in quarantine May 11, 1916, and was kept under observation thirty-three days before it was used in this experiment.

May 26, 1916, carabao 51 was used in an experiment on vaccination against rinderpest by the injection of desiccated virulent blood, but gave no reaction.

At no time during the period of observation did this animal present a high temperature or show any symptoms of sickness.

June 13, 1916, carabao 51 was inoculated with 10 cubic centimeters of blood from pig 300.

History of pig 300:

June 4, 1916: Inoculated with 10 cubic centimeters of virulent rinderpest blood procured from a carabao in Angono, Rizal Province.

June 9: First rise in temperature, registering, a. m., 39.2° C.; p. m., 40° C.

June 13: E. L.; bled to death, at which time the blood was procured to inject carabao 51.

June 19, carabao 51 presented a rise in temperature, registering, in the morning, 39.8° C.; in the afternoon, 40.7° C.

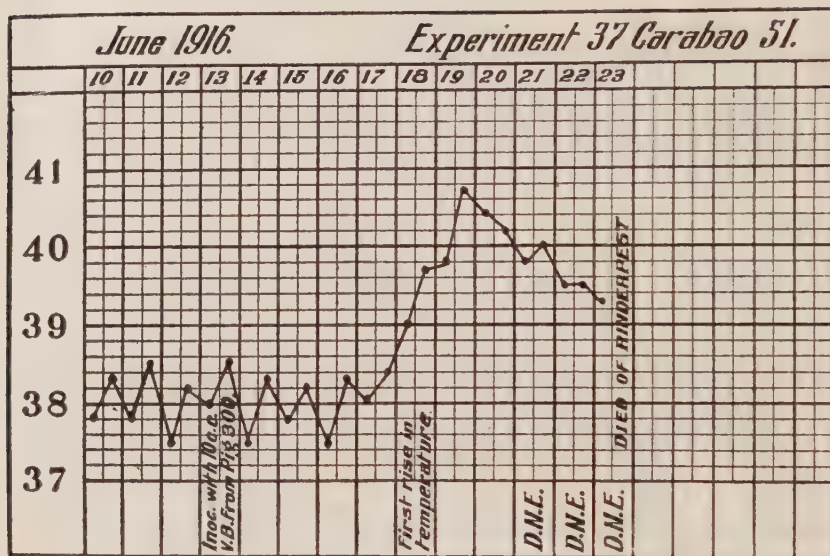


FIG. 9. Temperature chart of carabao 51 inoculated with blood from pig.

June 21-23 D., N. E.

June 23, died of rinderpest, presenting good lesions of that disease upon autopsy.

In summing up the results of this experiment, it will be noted that carabaos will contract rinderpest when injected with blood from pigs sick with rinderpest.

#### PIGS INOCULATED WITH BLOOD FROM CARABAOS SICK WITH RINDERPEST

The following experiments were designed to furnish information regarding the possibility of pigs contracting rinderpest when inoculated with virulent rinderpest blood from a carabao suffering with that disease.



EXPERIMENT 38

*Pig 299.*—Known history prior to the experiment: Native pig, 8 months old, purchased in Cavite Province in a locality which was free from rinderpest; it was brought to the laboratory and placed in quarantine May 30, 1916, and was kept under observation forty-eight days before it was used in this experiment.

June 2, E. L. This condition undoubtedly was caused by change in feed, as the animal did not present a high temperature or show any other symptoms of sickness during this period.

July 17, 1916, pig 299 was inoculated with 50 cubic centimeters of virulent rinderpest blood from carabao 53.

History of carabao 53:

July 1, 1916: Inoculated with 1.2 grams of dried virulent blood from carabao 54. This blood was dried in twenty hours and then kept in the ice box in a sealed tube for twenty-two hours.

June 10: First rise in temperature.

June 15: D., E. L.

June 16-19: D., N. E.

June 20: Died of rinderpest, presenting good lesions of that disease upon autopsy.

July 20, pig 299 developed a rise in temperature, registering, in the morning, 39.2° C.; in the afternoon, 40.2° C.

July 21-23, N. E.

July 24, E. L.

July 25-26, D., E. L.

July 27, died of rinderpest, presenting good lesions of that disease upon autopsy.

EXPERIMENT 39

*Pig 301.*—Known history prior to the experiment: Native pig, 7 months old, purchased in Cavite Province, brought to the laboratory and placed in quarantine May 30, 1916, and kept under observation forty-eight days before it was used in this experiment.

June 2, 3, and 4, this animal suffered from dietary disturbances, but immediately returned to normal. At no other time during this period did this animal present any symptoms of sickness.

July 17, 1916, pig 301 was inoculated with 50 cubic centimeters of virulent rinderpest blood from carabao 53. (For history of carabao 53 see experiment 38.)

July 19, pig 301 developed a rise in temperature, registering, in the afternoon, 40.3° C.

July 21-23, N. E.

July 24-27, E. L.

This animal gradually recovered.

In summing up the results of these two experiments, it will be noted that pigs will contract rinderpest when inoculated with virulent rinderpest blood from a carabao.

#### RINDERPEST TRANSMITTED FROM CATTLE TO PIGS BY MEANS OF THE CARETAKER

This experiment was designed to furnish information regarding the possibility of transmitting rinderpest from cattle sick

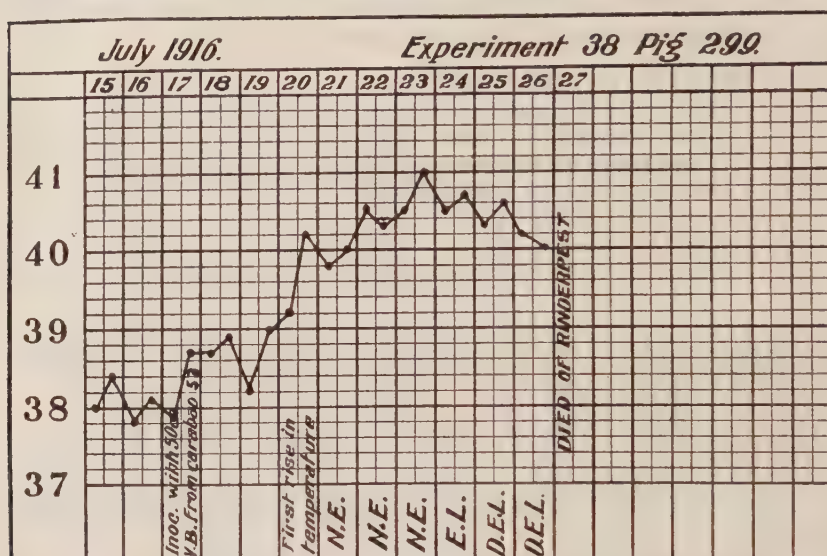


FIG. 10. Temperature chart of pig 299 inoculated with blood from carabao.

with rinderpest to pigs by means of the caretaker. The sick cattle and the susceptible pigs were cared for by the same man.

#### EXPERIMENT 40

On March 13, 1916, pigs 270, 271, 269, and 273 were placed in separate stalls in the same shed, where cattle sick with rinderpest were kept. These pigs were not kept in quarantine previous to their exposure and did not come in direct contact with the sick cattle, but were cared for by the same man, who took care of the sick cattle.

March 21, 1916, pig 270, native, three months old, presented a rise in temperature, registering, in the afternoon, 39.6° C.; this was eight days after the initial exposure.

March 26, D.

March 27-28, D., E. L.

March 28, died of rinderpest, presenting good lesions of that disease upon autopsy.

Pig 271, native, 5 months old, presented a rise of temperature March 28, 1916; this was fifteen days after the initial exposure. Its temperature on this day registered 40.4° C. in the afternoon.

April 3-5, N. E.

April 6, E. L.

April 7-8, N. E.

April 9, died of rinderpest, presenting good lesions of that disease upon autopsy.

Pig 269, native, 3 months old, presented a rise of temperature March 28, 1916; this was fifteen days after the initial exposure. Its temperature on this day registered 40.3° C. in the afternoon.

March 31, D.; morning temperature, 40.4° C.; afternoon temperature, 41.6° C.; this was the highest temperature recorded during the course of the disease.

April 1, 1916, D.

April 2, found dead in the morning. Presented good lesions of rinderpest upon autopsy.

Pig 273, native, 7 months old, presented a rise in temperature March 28, 1916, registering, in the afternoon, 40.1° C.; this was fifteen days after the initial exposure.

April 3-5, E. L.

April 6-7, D., E. L.

April 8-9, D., N. E.

April 10, D.

This animal gradually recovered.

In summing up the results of the exposure of these four pigs, it will be noted that pigs can contract rinderpest from cattle sick with that disease by means of the caretaker.

#### PIGS RECOVERED FROM RINDERPEST, AND THIS IMMUNITY TESTED TOWARD THAT DISEASE

The following experiment was designed to test the immunity of pigs to rinderpest after they had recovered from that disease. These animals were kept in the shed where the animals sick with rinderpest were placed, which gave them frequent exposures to animals sick with rinderpest in all stages of the disease.

#### EXPERIMENT 41

In this experiment pigs 94, 266, 267, 289, 291, 297, 301, 302, 155, and 154 were used.

*Pig 94.*—This animal was a Berkshire sow and had been hyperimmunized to hog-cholera virus and was later exposed to rinderpest by contact. It contracted and ran a mild course of the disease, recovering on December 5, 1914.

This animal was again exposed to rinderpest January 23, 1915, and these exposures have been continued up to date, covering a period of six hundred sixty-five days. During this time the pig has not shown any ill effects from these exposures, there having been no temperature or physical reaction.

*Pig 266.*—Recovered from rinderpest March 24, 1916. This animal was kept in the shed one hundred sixty days with animals sick with rinderpest and did not develop any ill effects from these exposures.

*Pig 267.*—Recovered from rinderpest April 2, 1916. This animal was kept in the shed one hundred fifty-one days with animals sick with rinderpest and did not develop any ill effects from these exposures.

*Pig 289.*—Recovered from rinderpest May 20, 1916. This animal was kept in the shed one hundred three days with animals sick with rinderpest and did not develop any ill effects from these exposures.

*Pig 291.*—Recovered from rinderpest May 13, 1916. This animal was kept in the shed one hundred ten days with animals sick with rinderpest and did not develop any ill effects from these exposures.

*Pig 297.*—Recovered from rinderpest June 28, 1916. This animal was kept in the shed sixty-four days with animals sick with rinderpest and did not develop any ill effects from these exposures.

*Pig 301.*—Recovered from rinderpest July 28, 1916. This animal was kept in the shed thirty-three days with animals sick with rinderpest and did not develop any ill effects from these exposures.

*Pig 302.*—Recovered from rinderpest June 8, 1916. This animal was kept in the shed eighty-four days with animals sick with rinderpest and did not develop any ill effects from these exposures.

*Pig 155.*—Recovered from rinderpest December 21, 1915. This animal was kept in the shed two hundred fifty-four days with animals sick with rinderpest and did not develop any ill effects from these exposures.

*Pig 154.*—Inoculated with 8 cubic centimeters of virulent rinderpest blood from bull 3995 on February 22, 1916. This an-



imal did not react and was kept in the shed one hundred ninety-one days with animals sick with rinderpest and did not develop any ill effects from these exposures.

In summing up the results of the exposures of the ten preceding pigs, it will be noted that there is apparently a permanent immunity conferred on them against rinderpest after recovering from that disease, or at least for practically two years, which is the average life of a hog used for food purposes.

#### PIGS RECOVERING FROM RINDERPEST AND LATER BECOMING INFECTED WITH HOG CHOLERA

The following experiment was designed to furnish information as to whether pigs that had recovered from rinderpest were susceptible to hog cholera.

##### EXPERIMENT 42

On May 15, 1916, pigs 293, 294, 295, and 296 were received at the laboratory and placed in quarantine.

May 17, these four animals developed symptoms which were later identified as those of hog cholera. On this date the four animals were transferred to shed No. 3 and placed in the same pen with pigs 265, 272, 273, and 292, which had recovered from rinderpest.

Pig 265 recovered from rinderpest March 22, 1916.

May 17, 1916, this animal was exposed to the four pigs sick with hog cholera; this was fifty-six days after it had recovered from rinderpest.

May 30, pig 265 presented a rise in temperature.

June 7-11, E. L.

June 12, died of hog cholera, presenting good lesions of that disease upon autopsy.

Pig 272 recovered from rinderpest April 10, 1916.

May 17, 1916, this animal was exposed to the four pigs sick with hog cholera; this was thirty-seven days after it had recovered from rinderpest.

May 30, pig 272 presented a rise in temperature.

June 10-16, E. L.

June 17-23, D., E. L.

June 23, died of hog cholera, presenting good lesions of that disease upon autopsy.

Pig 273 recovered from rinderpest April 11, 1916.

May 17, 1916, this animal was exposed to the four pigs sick

with hog cholera; this was thirty-six days after it had recovered from rinderpest.

June 1, 1916, pig 273 presented a rise in temperature.

June 10-15, E. L.

June 16-19, N. E.

June 20, D., E. L.

June 21-23, E. L.

June 23, this animal was killed and upon autopsy presented good lesions of hog cholera.

Pig 292 recovered from rinderpest May 9, 1916.

May 17, 1916, this animal was exposed to the four pigs sick with hog cholera; this was eight days after it had recovered from rinderpest.

May 30, 1916, pig 292 presented a rise in temperature. This animal ran a mild course, was killed on June 23, and presented a few lesions of hog cholera upon autopsy.

From the results obtained in the preceding experiment, it will be noted that pigs that have recovered from rinderpest are susceptible to and can die of hog cholera, which proves that there is no immunity toward hog cholera conferred upon a pig that has recovered from rinderpest.

#### PIGS HYPERIMMUNIZED TO HOG CHOLERA AND LATER CONTRACTING RINDERPEST

The following experiments were designed to ascertain whether pigs that had been hyperimmunized against hog cholera would contract rinderpest when exposed to cattle sick with rinderpest.

#### EXPERIMENT 43

*Pig 1.*—This animal received its last injection of 450 cubic centimeters of virulent hog-cholera blood on October 15, 1914.

November 16, 1914, pig 1 was exposed to bull 3845.

History of bull 3845:

November 9, 1914: Inoculated with 200 cubic centimeters of virulent blood from bull 3780.

November 11: Bull 3845 presented a rise in temperature.

November 11-16: D.

No. 17-18: D., N. E.

November 19: Died of rinderpest.

November 20, pig 1 presented a rise in temperature, registering, in the morning, 39.8° C.; in the afternoon, 41.9° C.

November 22-27, N. E.

November 28, E. L.

This animal's temperature gradually subsided to normal, and it made a complete recovery.

EXPERIMENT 44

*Pig 2.*—This animal received its last injection of 350 cubic centimeters of virulent hog-cholera blood on October 14, 1914.

November 9, 1914, pig 2 was exposed to bull 3780.

History of bull 3780:

November 21, 1914: Inoculated with 200 cubic centimeters of virulent rinderpest blood from bull 3838.

November 5: Bull 3780 presented a rise in temperature.

November 11: D., N. E.

November 12: E. L.

This animal gradually recovered.

November 13, pig 2 presented a rise in temperature, registering, in the morning, 40° C.; in the afternoon, 40.3° C.

November 16, afternoon temperature, 41.3° C.

The temperature of this animal was high until November 21, when it subsided to normal, and the animal made a prompt recovery.

EXPERIMENT 45

*Pig 4.*<sup>2</sup>—This animal received its last injection of 450 cubic centimeters of virulent hog-cholera blood on October 13, 1914.

December 1, 1914, pig 4 was exposed to bull 3847.

History of bull 3847:

November 28, 1914: Inoculated with 100 cubic centimeters of virulent rinderpest blood from bull 3873.

December 1: Bull 3847 presented a rise in temperature.

December 5-8: D., E. L.

December 9-10; D.

This animal gradually recovered.

December 8, pig 4 presented an afternoon temperature of 40.5° C.

December 9, the afternoon temperature was 41° C.

The temperature of this animal was high until December 15, when it subsided to normal, and the pig recovered.

EXPERIMENT 46

*Pig 5.*—This animal received its last injection of 400 cubic centimeters of virulent hog-cholera blood on October 14, 1914.

<sup>2</sup> Pig 3 was not used in this series of experiments.

November 23, 1914, pig 5 was exposed to bull 3846.

History of bull 3846:

November 16, 1914: Inoculated with 100 cubic centimeters of virulent rinderpest blood from bulls 3716 and 3845.

November 19: Bull 3846 presented a rise in temperature.

November 21: D., E. L.

November 22-23: D.

November 24-25: D., N. E.

November 26, died of rinderpest.

November 25, pig 5 presented an afternoon temperature of 41.1° C.

November 26-27, the afternoon temperature was 41.7° C.

November 28, N. E.

November 29, D., N. E.

November 30, E. L.

December 1, E. L.

This animal's temperature returned to normal December 6, and the pig recovered.

EXPERIMENT 47

*Pig 6.*—This animal received its last injection of 300 cubic centimeters of virulent hog-cholera blood on October 14, 1914.

November 16, 1914, pig 6 was exposed to bull 3846. (For history of bull 3846, see experiment 46.)

November 19, pig 6 presented a morning temperature of 39.7° C.; and an afternoon temperature of 41.7° C.

November 23-27, N. E.

November 28-30, E. L.

December 1, E. L.; this animal was killed on this date.

EXPERIMENT 48

*Pig 7.*—This animal received its last injection of 450 cubic centimeters of virulent hog-cholera blood on October 15, 1914.

November 12, 1914, pig 7 was exposed to bull 3842.

History of bull 3842:

November 7, 1914: Inoculated with 100 cubic centimeters of virulent rinderpest blood from Pampanga Province.

November 13: Bull 3842 presented a rise in temperature.

This animal ran a rather mild form of the disease and recovered.

November 16, pig 7 presented a morning temperature of 39° C. and an afternoon temperature of 41.1° C.

November 17, the afternoon temperature was 41.2° C.

November 21, this animal's temperature had subsided to normal, and the pig made a rapid recovery.



In summing up the results of these experiments, it will be noted that pigs that have been hyperimmunized to hog cholera can contract rinderpest when exposed to cattle sick with rinderpest. These results prove that there is no immunity conferred against rinderpest by the hog-cholera virus, although the animals ran a milder course of the disease than has been usually witnessed in the laboratory. This condition may be considered from two viewpoints: First, that possibly the strain of rinderpest virus was not very virulent; and secondly, that the hyperimmunization against hog cholera may have increased the resistance toward rinderpest.

#### CONCLUSIONS

1. From the results obtained in experiments 1 to 10, inclusive, it is proved that pigs can contract rinderpest when exposed to cattle sick with rinderpest. The disease thus contracted by pigs may terminate in death, unthriftiness, or complete recovery.

2. From the results obtained in experiments 11 to 13, inclusive, it is proved that pigs can contract rinderpest when exposed to pigs sick with rinderpest.

3. From the results obtained in experiment 14 it is proved that a pig can contract rinderpest from pigs sick with rinderpest by means of the caretaker.

4. From the results obtained in experiments 15 and 16 it is proved that pigs can contract rinderpest when inoculated with blood drawn from pigs sick with rinderpest.

5. From the results obtained in experiments 17 to 20, inclusive, it is proved that pigs can contract rinderpest when inoculated with blood drawn from cattle sick with rinderpest.

6. From the results obtained in experiment 21 it is proved that a pig can contract rinderpest when inoculated with mixed blood drawn from cattle and pigs sick with rinderpest.

7. From the results obtained in experiment 22 it is proved that a pig can contract rinderpest when it is drenched with blood drawn from carabaos sick with rinderpest.

8. From the results obtained in experiments 23 to 28, inclusive, it is proved that cattle can contract rinderpest when exposed to pigs sick with rinderpest. However, cattle do not contract the disease very readily by this method, and the exact reason for this has not been ascertained at the present writing.

9. From the results obtained in experiments 29 to 32, inclusive, it is proved that cattle can contract rinderpest when inoculated with blood drawn from pigs sick with rinderpest.

10. From the results obtained in experiment 33 it is proved that cattle can contract rinderpest when inoculated with mixed blood drawn from pigs and cattle sick with rinderpest.

11. From the results obtained in experiments 34 and 35 it is proved that cattle can contract rinderpest when drenched with urine collected from pigs sick with rinderpest.

12. From the results obtained in experiment 36 it is proved that carabaos can contract rinderpest when exposed to pigs sick with rinderpest.

13. From the results obtained in experiment 37 it is proved that carabaos can contract rinderpest when inoculated with blood from pigs sick with rinderpest.

14. From the results obtained in experiments 38 and 39 it is proved that pigs can contract rinderpest when inoculated with blood from carabaos sick with rinderpest.

15. From the results obtained in experiment 40 it is proved that pigs can contract rinderpest from cattle sick with rinderpest by means of the caretaker.

16. From the results obtained in experiment 41 it is proved that pigs remain immune to rinderpest after their recovery from that disease for at least six hundred sixty-five days and undoubtedly during the rest of their existence.

17. From the results obtained in experiment 42 it is proved that pigs that have recovered from rinderpest are susceptible to and may die from hog cholera, which shows that there is no immunity against hog cholera conferred upon a pig that has passed through an attack of rinderpest.

18. From the results obtained in experiments 43 to 48, inclusive, it is proved that pigs that have been hyperimmunized to hog cholera are susceptible to rinderpest when exposed to cattle sick with rinderpest. Although the pigs used in these experiments did not run as severe a course of the disease as the average pig, there is a possibility that the strain of virus with which they were infected was not of the most virulent type. This condition is frequently noticed in rinderpest in cattle. One strain may cause a high mortality, while another may cause a comparatively low mortality. Thus it is rather difficult to state definitely that the hyperimmunization to hog cholera was of any benefit to the pigs when they were exposed to rinderpest.

19. In summing up the results of all of these experiments, it will be noted that cattle, carabaos, and pigs vary but slightly in susceptibility to rinderpest and that the disease can be transmitted practically as readily from one kind of animal to the other as among the individuals of a single species.

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## ILLUSTRATIONS

[Plates lent by the Bureau of Agriculture.]

### PLATE I

A group of pigs sick with rinderpest.

### PLATE II

FIG. 1. A pig in the last stages of rinderpest.

2. A pig in the last stages of rinderpest, possessing marked eye lesions.

### TEXT FIGURES

FIG. 1. Temperature chart of pig 240 exposed to bull.

2. Temperature chart of pig 212 exposed to pig.

3. Temperature chart of pig 219 inoculated with blood from pig.

4. Temperature chart of pig 241 inoculated with blood from bull.

5. Temperature chart of cow 3995 exposed to pig.

6. Temperature chart of bull 3839 inoculated with blood from pig.

7. Temperature chart of bull 3993 drenched with urine from pig.

8. Temperature chart of carabao 57 exposed to pig.

9. Temperature chart of carabao 51 inoculated with blood from pig.

10. Temperature chart of pig 299 inoculated with blood from carabao.





PLATE I. A GROUP OF PIGS SICK WITH RINDERPEST.





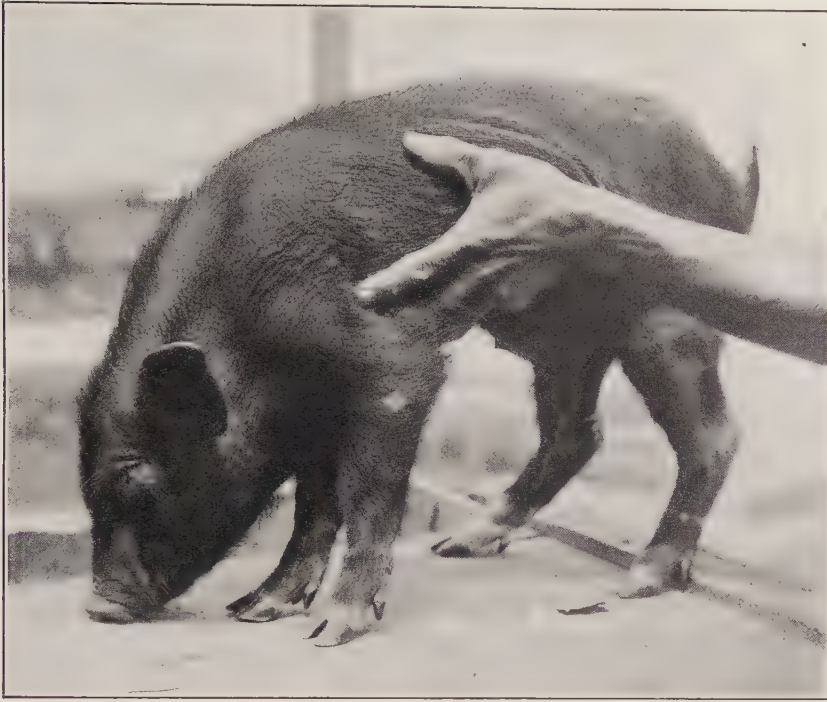


Fig. 1. A pig in the last stages of rinderpest.



Fig. 2. A pig in the last stages of rinderpest, possessing marked eye lesions.



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CULTIVATION OF A PATHOGENIC FUNGUS WHICH EXHIBITS  
BOTRYOID AND LEUCOCYTELIKE PARASITIC FORMS <sup>1</sup>

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SIX PLATES

In the Philippine Islands, as in other tropical regions, the material available for the study of fungus infections is more abundant than in most other parts of the world. Not only are mycotic lesions very common, but it appears that there are here to be found new types of infectious fungi, some of which, at least, present features that have rarely or never been described as occurring among such organisms.

The purpose of the present communication is to report a recently isolated fungus which possesses a remarkable range of morphologic variation and which is, perhaps, related to, or possibly itself is, the etiological factor of certain conditions whose cause is not understood. In one of its phases this organism produces minute endospores, somewhat like those of bacteria, but quite different from any of the recognized spores of fungi. Another feature is the resemblance of forms which the fungus may assume to certain body cells, particularly the lymphoid and plasma cells. These leucocytelike parasites occur in the active lesions, and in certain cultures their development can be followed. These and other features that the fungus presents in its various phases and modes of development appear not to be peculiar to this fungus alone and seem of considerable interest not only from the standpoint of the pathologist, but also from that of the mycologist.

<sup>1</sup> Received for publication September 1, 1916.

The case from which this fungus was isolated was encountered among a number of leper suspects, among whom unfamiliar types of skin infections are occasionally to be seen. This individual, who presented multiform lesions of several types, proved to be nonleprous; he was, however, kept under observation for some time. The principal features of the case, so far as they can be determined, are as follows:

#### CASE HISTORY

The patient is a male Filipino (Tagalog), laborer, aged about 30 years, resident in Manila for some years. He says that in July or August, 1915, an ulcerative lesion appeared below the left elbow. This healed, but soon reappeared, involving a wider area, beyond which it spread slowly. At about the same time a number of similar spreading lesions appeared simultaneously on other parts. The patient experienced a chill at the time of onset, but thinks that he had no fever. These lesions spread over considerable areas as evidenced by the cicatrices, some of which are of considerable thickness and resemble keloids (fig. 5). About four months later numerous small, scattered lesions began to appear, said to have directly followed a three-day course of some medication.

Upon examination (June 6, 1916) the lesions are seen to be very numerous and varied. On the legs and feet there seems to be little normal skin left. The hands and forearms are also very much involved.

Small, early lesions are present in great numbers over the back (fig. 1), forearms (fig. 4), and legs and to a less extent on the arms, thighs, and shoulders. These are very superficial, erythematous, slightly raised, and somewhat indurated areas, irregular in size and outline, in which are numerous fine papules usually pinhead-sized or smaller. These are not tender on pressure. In and immediately about the areas, except over the papules, the epidermis is pigmented, sometimes to a deep brown. It was probably the peculiar appearance of these small lesions, together with a nodular induration of the lobes of the ears (fig. 2), that had caused the patient to be submitted for examination as a suspected leper.

The small lesions seem more commonly to subside, leaving slight scars or disappearing completely. Over the wrists and hands, feet and ankles, the elbows and the knees, and even on the upper margins of the ears (fig. 2), numbers of them have progressed to form rather prominent verrucae, which vary in color from white to a dark gray. These apparently develop by a low-grade inflammatory hyperplasia.

A more striking type of lesion, comparatively acute, which apparently originates with the common small lesion, is a relatively large frambœsial nodule (fig. 3), which extends over a part or the whole of a papular area, often encroaching on the adjacent skin. These lesions are abruptly raised and usually oval, measuring from 1 to 2 centimeters in width and from 1.5 to 2.5 or 3 centimeters in length. The covering epidermis is thin, shiny, and transparent, and thin scales usually form upon it. It is apparently easily injured. Beneath it, in the more or less dark gray to purplish gray newly formed tissue, are seen red pinhead-sized points suggesting vascular spaces and others which are pale gray to yellowish, apparently due to necrosis or suppuration. A somewhat similar acute lesion sometimes develops by recurrence in scar tissue (fig. 5, indicated).



These nodules upon incision usually appear much less than normally sensitive. The tissue is very friable and tears readily. Incised wounds heal promptly and cleanly unless secondarily infected. Such an infection is apparently not uncommon and results in more or less persistent ulcers, which are shallow, do not increase in size or do so slowly, exude constantly, and heal reluctantly. Excess scar formation has followed as a rule.

During a period of two months' observation the changes that took place were of minor importance. As a whole, more and more of the skin surface was being encroached upon. The patient's general condition was fairly good, though he was slightly anæmic and below normal in strength.

#### STUDY OF ORGANISM

At various times smears and cultures were made, and on three occasions tissue was excised for study. After several unsuccessful attempts to cultivate it, the fungus was recovered in its free-growing form. In the earlier cultures there were isolated from certain acute, unbroken lesions a nonhæmolytic *Streptococcus pyogenes* and a delicate diphtheroid. In view of the findings of Rosenau<sup>(9)</sup> in erythema nodosum, these were studied somewhat fully, but they proved not to be significant except as secondary invaders.

In the earlier cultures the fungus was not recognized, though certain of its forms were later found to have been present in some of the preparations from these cultures.

In describing the various appearances of the organism, it seems advantageous to apply tentatively selected names to forms which, it appears, are not covered by any accepted terminology. These are *a*, the "basic parasitic form," a simple, usually round, densely staining, more or less nucleuslike body, from which other forms develop; *b*, the "entire-cell form," which is evolved by the development of a distinct protoplasm about the basic form; *c*, the "spore-granules," produced under conditions of semiparasitism, which are given this qualified designation because they are very different from the usual spores of fungi and, to a less extent, from common bacterial spores; and *d*, the "gelatinous body," another semiparasitic form, which is a peculiar cultural development of the basic form.

#### HISTOPATHOLOGY

The tissue forms of the parasites were first found in smears and sections of the soft, granular tissue of a fairly recent, uncomplicated lesion. A small piece was removed and placed in Zenker's fluid; cultures were made from the cut surface. In these cultures there later appeared the first recognized multiplication of the fungus.

The tissue studied was from the nodule proper and is com-

posed of epidermis and newly formed inflammatory tissue. None of the cutaneous gland structures are present. The epidermis is intact, though it is somewhat thin and irregular and, at a few points, as shown in figs. 8 and 9, is invaded by scattered parasites. Throughout the sections vascular channels are very numerous. Here and there, usually close under the epidermis, are areas so vascular and blood-filled as to suggest hemorrhage (fig. 6). In the large, irregular, more or less cavernous spaces, which are usually lined with endothelium and filled with normal erythrocytes, are numbers of leucocytes and apparent forms of the fungus. The lymphatic channels are numerous and prominent in the deeper layers of the tissue. Everywhere there is evident an extensive leucocytic infiltration, particularly in and about the vascular areas (fig. 7). Here are polymorphonuclear leucocytes and, more numerous, cells of the "round-cell" types. In occasional small, active areas are numerous large endothelial leucocytes, occurring usually within blood channels, as in figs. 7 and 10. Most of them, actively phagocytic, contain numbers of parasite bodies. Many of the parasitic cells, even after prolonged study of the cytology of the lesions, are impossible of differentiation from body cells.

In the less active foci are not a few eosinophiles, which have always been observed in the smears from this type of lesion. There is no evidence of pigment deposit in the sections.

By comparing the bodies seen here with certain of those seen in preparations from cultures, the apparent forms of the parasite in the lesion may be determined.

#### FORMS OF ORGANISM IN TISSUE LESION

The basic forms are simple, small, round, sometimes oval and occasionally crescentic or signet-ring-shaped bodies which, unless degenerated (as in fig. 10), stain intensely by Gram's and general nuclear stains. Occasionally one may find within a phagocyte several organisms which are bean-shaped. Some are found to be slightly acid-fast. The smaller vary in size from mere granules, hardly larger than the ordinary staphylococcus (figs. 8, 9, and 14), to bodies 3 or 4 microns in diameter (figs. 8 to 14). These show no apparent structural differentiation. Practically all of the phagocytized parasites and the smaller of those that are free are of this type. The entire-cell form is produced by the development about the free-lying basic forms of protoplasmic envelopes (see especially figs. 8, 9, 13, and 14). In sections these bodies often measure from 5 to 6 microns in diameter.

*Multiplication.*—Having attained their maximum size, the entire-cell forms sometimes undergo a simple segmentation, during which the nuclear mass becomes irregular in outline (fig. 10) and is broken up to form botryoid groups of from 5 to 10 small, round, basic forms of different sizes, the individuals of which are ultimately liberated by dissolution of the protoplasm (figs. 8 and 14, *c*). There is no regularity in the number of small forms produced. The segmented parasite in fig. 14 is of unusually large diameter. These groups are not numerous and have been found only in the superficial, more active areas. More common than this segmentation is multiplication by the simple fission of a nuclear body into parts, equal or unequal. This may occur even in the smaller parasites (14, *b*) and occasionally is seen even among the phagocytized bodies.

In the active foci the free-lying forms are particularly numerous, occurring in blood and lymph spaces and among the tissue cells. Infrequently they are seen, as in figs. 13 and 14, invading areas of the epidermis without cellular reaction.

As the parasites do not stain differentially, their recognition both in tissue sections and in smear preparations is dependent in part upon morphology, the outline of the nuclear body usually being strikingly clear-cut, and particularly upon their intensity of staining. In view of their frequently close resemblance to lymphoid and plasma cells and of the intensity with which injured tissue-cell nuclei stain, this is at best an uncertain criterion. The more solid bodies retain Gram's stain more intensely than do tissue nuclei, but a clear differentiation is not possible. There has nowhere been found evidence of capsule formation, the material which develops about the free-lying forms being distinctly protoplasmic in its staining reaction.

#### CULTURAL FEATURES

Slight multiplication of this organism in cultures on solid media seems always to occur, provided tissue elements are present, but on all except a few media it soon ceases. In smears from such cultures, which appear sterile, the forms that result from this brief multiplication may be easily passed over without recognition, particularly after staining by Gram's method (figs. 15, 16, and 17). Many of the appearances which it may assume are so dissimilar that some of the forms might well pass unrecognized in the search for others. The principal modes of development as they have been observed will be described separately.

#### DEVELOPMENT OF PARASITIC FORMS ON BLOOD-SMEARED AGAR

If material obtained by scraping an incised, active lesion be



planted in a drop or two of blood, whole or defibrinated, smeared on an agar slant, there ensues a course of development some features of which are not to be seen under other conditions.

The homogeneous basic forms, which are slightly blue after Giemsa's stain, enlarge somewhat and stain more deeply. Sometimes the entire-cell form becomes prominent (fig. 18).

*Spore-granules.*—Within the "nuclear" body of the enlarged parasite, whether it be endowed with protoplasm or not, there develop a few extremely small, lighter points, due to the earliest appearance of the small hyaline spore-granules (fig. 19, upper parasite). The color of the cell now becomes distinctly purplish in Giemsa-stained preparations, more closely resembling a degenerating leucocyte nucleus. The spore-granules enlarge and become more numerous (fig. 19, lower parasite), appearing as minute, round, oval, and rod-shaped bodies.

The outline of the containing cell soon becomes indefinite, and the whole appears soft (fig. 20). Smears occasionally show groups of the spore-granules that are compactly massed (fig. 21). When liberated the spore-granules usually become indistinguishable in smear preparations, though they may be present in large numbers (fig. 22). After Gram's stain they retain the safranin counterstain and may be tentatively identified by their higher refraction. Giemsa's stain does not color them as a rule. After Loeffler's blue the larger are often easily discernible by their clear, light, refractile blue.

The formation of these spore-granules seems an extremely simple process. There is no definite segmentation, nor is there any apparent mechanism governing the number or size. They appear much as might crystals separating out of a protoplasmic mass and occur only within the nucleus until this is no longer demarked. They have not been observed in the tissue sections. While they seem to be produced for multiplication only, comparatively very few are capable of development under the conditions obtaining. In the free blood of these cultures a few (as in the center of fig. 22) enlarged into typical basic forms. In occasional cultures the viable granules, before developing into the leucocytelike bodies and cells, become rod-shaped or slightly curved, often appearing as small bacilli (fig. 23). Prominent in most of these half-grown and larger organisms is the appearance of one or two small metachromatic granules, often placed as bars across the organism. This was the first form to be recognized as developing in the cultures and at first was misunderstood. The more mature cells are usually oval (figs. 24 and 25), and after Giemsa's stain the protoplasm presents a clear,



delicate, robin's-egg blue and the metachromatic granules and masses, usually of a clear red, are prominently in contrast. This type of cell develops into the leucocytelike form, with or without protoplasm.

#### DEVELOPMENT OF PARASITIC FORMS ON MEDIA WITHOUT FREE BLOOD

In tissue scrapings planted on ordinary media without smeared blood there occur forms of early multiplication, most of which might well be mistaken for products of degeneration of inoculated tissue. In smears stained by Gram's there is often to be seen only amorphous material containing a few Gram-positive granules and small, round, or oval bodies (fig. 15). There often are more or less numerous small masses (8 to 12 microns in length), which may be either body or parasite cells (fig. 16) that, because of their rather hyaline, amorphous appearance, may be mistaken for fragments of *débris*. In some of these cultures the large gelatinous forms appear, but these are not constantly found.

Multiplication of the organisms in these cultures persists only so long as there is tissue substance present; consequently the organisms soon die out.

#### DEVELOPMENT IN PLANTED TISSUE BITS

Here the parasitic forms sometimes enlarge and quickly produce hyaline spore-granules. Often, however, they are transformed into the very much larger, gelatinous-appearing masses noted, which have developed fully only within pieces of planted tissue. These may be unstained and scarcely recognizable after Gram's stain (fig. 17).

*Gelatinous bodies.*—In an occasional preparation one may trace the transition of the basic form to small gelatinous bodies (fig. 26) and the enlargement of these. In some cultures those of medium size seem firm and stain deeply, as though the contents were thick and gelatinous, though in more instances they appear to be softer.

Under some conditions the gelatinous bodies develop vast numbers of the small, hyaline spore-granules. At other times the spore-granules and deeply staining basic forms, often very small, develop simultaneously. The latter at times appear in large numbers, with or without the hyaline granules. In fig. 28 is a group of basic-form bodies which developed from such a gelatinous mass as shown in fig. 27. In fig. 29 is an unusual group of closely packed "gelatinous" forms, now thin and vesicular, all of which contain numbers of the basic granules and bodies.

The most favorable condition for this development is cultiva-

tion on banana agar at tropical room temperature, about 30°.² On such media the basic masses develop extensively and irregularly, assuming bizarre forms (fig. 30). These represent the earliest attempts of the parasitic forms to produce a mycelium. They are to be seen after several days of cultivation. The deeply staining figures become larger and more irregular from day to day and finally grow out of the containing cell and become definitely mycelial. After fifteen days or more, what remains of the tissue bit within which this development occurs appears whitish and delicately fuzzy with hyphous growth. Smears now show mycelium and great numbers of calcium oxalate crystals.

One feature of smears from banana cylinders is the occurrence of vesicular bodies (fig. 31), which might be thought to develop from the gelatinous form of the parasite. They usually are, in reality, cells of the banana pulp and are sometimes of interesting appearance. One podlike cell shown in fig. 32 had ruptured; one with an unusually delicate wall is shown in fig. 33 doubled upon itself. It is possible that the body in fig. 30 is such a banana cell within which basic parasitic forms of the fungus are developing, though this cannot be asserted.

#### CULTURAL FEATURES OF MYCELIAL FORM

Once the mycelial type of growth has been established, subcultures grow rapidly and very luxuriantly on practically all media, often growing up along the glass of the test tube within a few days.

*On solid media.*—The downy, pure white growth is a tangled mycelium with many long, straight hyphæ radiating from the inoculation point (figs. 37 and 38). After a day or two it becomes tangled, appearing as if small fluffy snowflakes had become entangled in the loosely interwoven mass (fig. 36).

On glycerin, dextrose, and maltose agars the growth is particularly rapid and luxuriant. On potato and banana plugs it grows less luxuriantly, but on banana agar it develops very rapidly (compare figs. 37 and 38). Any of these cultures, when a few days old, have a typical "yeasty" odor which is striking. This I have never noted in cultures of any other mycelial fungus.

² Banana media, whether cut cylinders or banana pulp (10 per cent or more), in ordinary agar or bouillon, must be sterilized intermittently (Arnold sterilizer) and not autoclaved. The use of this material was suggested to me by Dr. J. A. Johnston, of this institution, who knew of its previous use here by Dr. F. Schmitter, of the United States Army Medical Corps, in the study of pathogenic fungi. In what form he used it has not been learned, as no report of such work has appeared under his name.

After several days the aërial growth for the most part collapses and the surface appears dry and dusty over a compact, firm surface layer. The agar itself becomes gradually clouded by down growth of the mycelium and assumes a yellow to light brown tint.

*In fluid media.*—In the depths of ordinary bouillon the fungus grows in loose, flocculent masses. In glycerin bouillon, whether 0.5 or 10.0 per cent, the growth is very slow, occurs in much smaller, more compact white masses than in other bouillons, and is always deep in the medium.

In most fluid cultures the greater part of the growth is on the surface where, in a few days, a thick pellicle is formed. The upper surface is at first deeply covered with fine aërial hyphæ which, as on agar, soon collapse (fig. 40). In a few weeks the bouillon assumes a deep amber color.

Growth is by far most abundant on banana bouillon with or without glycerin (fig. 39). The layer is firm, the under surface is smooth, and when torn there appears below the superficial mycelium a distinct membrane, about 1 millimeter thick, which is sharply demarked, firm, homogeneous, and yellow.

Growth is markedly greater at room than at incubator temperature. Anaërobiasis is also very unfavorable to active growth, often inhibiting it completely.

#### MORPHOLOGY OF MYCELIAL FORM

*On solid media.*—In the rapidly growing cultures the young hyphæ are rather thin and long and are branched at rather infrequent intervals. Septation is seen only in old filaments (fig. 43). In the aërial hyphæ vacuoles develop (fig. 41), and the protoplasm is reduced to narrow transverse bands somewhat suggestive of septa which determine the points at which the filament separates into individual cells. This process of maturation and dissociation may be complete in two or three days.

In the separated elements, as in figs. 34 and 35, there sometimes is evident a tendency to polar accumulation of the protoplasm. Hyaline granules often appear, and large and small metachromatic granules are common. After a time some of these entire segments become hyaline (fig. 42).

Upon transplanting these elements to fresh media, development usually begins within three or four hours by a lateral pseudopodic offshoot. Sometimes two and, rarely, three of these develop from a single mother cell, which becomes vacuolated and remains (as in figs. 41 and 42) in direct connection with the growing hyphæ.



*In fluid media.*—In plain bouillon the mycelium which is in the depths remains filamentous, though the aërial growth undergoes separation. After a few days the protoplasm of most of the submerged filaments condenses to form small, highly refractile granules (figs. 43 to 45). At the same time there may be formed short, irregular hyaline filaments which are faintly yellow, to massing of which the brittleness and deep yellow color of the tough yellow membrane which develops on banana bouillon are due. Rarely in old bouillon cultures there appear large chlamydospores (figs. 44 and 45), which develop terminally on apparently undifferentiated hyphæ.

A constant feature, even in young cultures, is the production of crystals which are usually flat, six-sided, and faintly yellowish, though under some conditions they have the typical "envelope" forms of calcium oxalate. These crystals are sometimes present in great numbers.

#### PATHOGENICITY FOR ANIMALS

As yet no extensive study has been made of the lesions which develop after inoculation of cultures of this mycelium into laboratory animals. Suffice it for the present to say that lesions not simply referable to the presence of the foreign material and which contain typical tissue forms of the parasite develop upon subcutaneous inoculation of fairly large doses. One rabbit which was inoculated intravenously died after twenty hours. There was found a peritonitis and an early plastic pericarditis, and in the exudates were great numbers of a modified entire-cell form of the organism. In some of the cultures from this animal on ordinary media the mycelial growth did not develop, while in others it appeared after three days.

#### SUMMARY AND DISCUSSION

From a case of mycotic dermatitis presenting multiform, widely distributed lesions, there has been isolated on special media, after several unsuccessful attempts, a fungus which is thought not to have been previously described. The present description is based on the observations made during its isolation from the only case of this nature yet encountered and during the few months since its recovery. Intensive study of numerous cultures has clearly demonstrated its life history under the various conditions obtaining.

In the saprophytic, free-growing state it forms on culture media a firm surface layer with abundant soft, white, loosely interwoven superficial growth. Under the conditions which have



obtained, it is nonfructifying, is septate in a minority of filaments, and the aërial hyphæ separate into short cylindrical cells which may later become oval, suggesting the yeastlike fungi. It grows luxuriantly on most laboratory media, and all cultures develop a strong "yeasty" odor.

Under conditions of animal-tissue parasitism the fungus is extremely modified, appearing primarily as solid, usually round bodies of nuclear material, most of which are free in tissue spaces. These parasites are often identified with difficulty, and in many preparations may be overlooked. They usually multiply by a simple fission to form two bodies, though in some cases, having attained maximum size, a parasite will undergo irregular multiple fission of the nuclear portion to produce botryoid groups of small bodies of different sizes which are ultimately liberated by dissolution of the protoplasm.

In different conditions of artificial cultivation the basic tissue forms may undergo various changes. In cultures containing free blood or tissue elements, that is, in what may be considered semiparasitic environment, they continue to multiply as parasites, and on no ordinary medium is the saprophytic habit of growth recovered. In these cultures, however, often after developing a protoplasm, they undergo modification to the extent that they differentiate within themselves numbers of a peculiar, very small, hyaline spore-granule, this process being inaugurated in the nuclear portion which later loses its identity. Under favorable conditions some of these spores enlarge, become deeply staining, and repeat the cycle.

This type of endosporulation is, so far as I have been able to learn, without precedent. The spore-granules are different from bacterial spores in size, regularity, and the capability of withstanding unfavorable conditions except, perhaps, drying. Furthermore, but a small proportion of them has proved viable even under the most favorable circumstances. They are still more unlike any of the many types of spores familiar to mycologists. Hyaline granules seen in fungi—probably of the nature of ordinary lipoid and other protoplasmic granules (as in figs. 44 to 46)—have often been called endospores, though their germination has not been observed. In the described fungus the spore-granules are produced in an entirely different manner and have developed only under conditions of semiparasitism.

The large gelatinous bodies which develop in some cultures also seem to represent a condition of semiparasitism and are intermediate between the tissue forms and the free-growing

fungus, since from them either of these may develop. The more or less gelatinous content of these large, sometimes almost cystlike bodies may produce: *a*, spore-granules alone; *b*, spore-granules and basic forms, larger and smaller; or *c*, basic forms alone. Under the most favorable conditions (in my experience only on properly made banana media), the basic forms thus produced within these bodies enlarge, become irregular in shape, and after two weeks develop the saprophytic mycelial fungus. This gelatinous body seems also to be very unusual, as I have found no mention of similar bodies in descriptions of other fungi.

A striking feature of the study of this fungus has been the difficulty encountered in converting the parasitic to the saprophytic form and the extreme contrast which these forms present. The difficulty of cultivation was not due to the lack of viability of the parasitic forms, since they multiplied fairly readily in certain artificial environment, but was simply due to the inability of the parasitic forms to readapt themselves to saprophytism. The real nature of the fungus and the significance of its tissue forms could not have been established had not the utilization of banana media accomplished its cultivation in free-growing form.

Little can be said of the botanical classification of the organism, pending further investigation of its relation to certain other fungi, particularly in the manner of its endosporulation. It seems likely that it will prove closely related to *Monilia*, though it is distinct from that genus.

The multiplicity of forms that this fungus may assume is unusual for a pathogenic fungus, though the forms themselves probably may be produced by other infectious fungi. Bodies similar to those here called the basic parasitic forms have been not infrequently described in other mycotic lesions, though in some instances they are described as "spores," while in most instances they have been thought to be products of degeneration of tissue and fungus cells. They appear, on the contrary, to be the basic multiplication form which a number of different fungi—how many cannot be stated—assume in adapting themselves to the parasitic rôle.

Fungi, in adapting themselves to conditions of animal tissue-cell metabolism, undergo extreme modification in structure and form. That these vegetable cells, so modified physically and chemically as to be able to abstract nutriment from the more dense body fluids and to utilize such material, may assume characteristics that make it almost impossible clearly to dis-

tinguish them by morphology or by staining reactions from the simpler types of body cells does not seem remarkable. It is, however, a fact that has not been generally recognized.

The clinical features of the described infection cannot be broadly discussed on the basis of a single case. An interesting feature that may be noted, however, is the wide distribution of a great number of small lesions, indicating a hematogenous dissemination. This is emphasized by the histology of the active lesion, in which are large vascular spaces many of which contain parasitic bodies. While tissue sections containing simple parasitic forms might be easily passed over without remark, the parasite-filled phagocytic cells and the botryoid clusters of various-sized bodies, which in my sections have been found only in the more active areas, are striking.

While no case clinically similar has been found in the literature, certain descriptions of the condition known as "botryomycosis hominis" and a few of those called, perhaps erroneously, "tropical inguinal granuloma" indicate some similarity of histologic findings with those of the present case.

In the small granulomatous skin tumors, first described by Poncet and Dor,<sup>(8)</sup> there are often to be seen small groups of bodies apparently like those here described. Whether rightfully or not, this condition has in recent years been classed by many authors as a staphylococcal granuloma. Very different, however, are the extensive lesions described as botryomycotic by Archibald<sup>(1)</sup> from the Sudan and by Castellani<sup>(3)</sup> from Ceylon. These are sometimes tumorlike and may even necessitate amputation of entire parts of the body. The former author found in sections groups of large and small bodies, free and in endothelial leucocytes, and also described and depicted a protoplasmic rim about some of the free bodies.

The typical "tropical inguinal granuloma" as described by Donovan,<sup>(5)</sup> Carter,<sup>(2)</sup> Conyers and Daniels,<sup>(4)</sup> Wise,<sup>(11)</sup> Flu,<sup>(6)</sup> and others is evidently a distinct infection, thought by many to be protozoic. However, certain cases that have been given this diagnosis may be of different nature. Such, for instance, are the three cases in American negroes reported by Grindon,<sup>(7)</sup> in sections from which the peculiar bodies of Donovan and of Carter were not found. In an illustration accompanying his article (his figure 6) is shown a photomicrograph of a section in which are discernible, both within the epidermis and in the connective tissue, many small, compact bodies and "cells" which, as well as can be made out, seem indistinguishable from any of the



parasite bodies of the similar section in fig. 9 herewith. Strangman<sup>(10)</sup> claims to have isolated a complex fungus from cases of granuloma pudendi.

Obviously one cannot more than suggest the possibility that, so far as the microscopic appearances of the described lesions are concerned, some of these conditions are in some ways similar to the infection in this case.

#### CONCLUSIONS

From a case of a peculiar chronic skin infection there has been isolated an apparently hitherto undescribed fungus. Some of its parasitic forms are very difficult or impossible to identify in the lesions, as they have the ordinary staining reactions of nuclear material. In cultures, forms frequently develop that are easily overlooked as not significant. It was obtained free-growing with considerable difficulty, having regained its mycelial habit only on banana media, after which it grew luxuriantly.

Phases of its morphology having reference to the described basic form, entire-cell phase, gelatinous and vesicular-body formation, and reproduction by endosporulation are features not generally recognized as occurring among fungi. It is believed, however, that certain familiar fungi, apparently distinct but possibly related, may under similar conditions exhibit certain of these characteristics.

The histopathological features of the described infection suggest its relation to the so-called botryomycosis hominis and possibly to certain cases, described as granuloma inguinale.

The classification and the name of this new fungus are left in abeyance, to await the results of other studies now under way.

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## ILLUSTRATIONS

### PLATE I

- FIG. 1. Numerous small lesions on back, with one frambœsial nodule.  
2. Showing the deep infiltration of the lobe of the ear and the verrucous lesion of the margin.  
3. Closer view (slightly larger than natural) of nodule in fig. 1.  
4. External surface of right arm, showing numerous active lesions and edge of an extensive scar on the posterior surface.  
5. Extensive keloidal cicatrix on left elbow. An active lesion later developed at the point indicated.

### PLATE II

- FIG. 6. Low magnification of granulomatous tissue from a typical active nodule, after Mallory's connective-tissue stain. The more active lesions under the epidermis photograph black because of great numbers of fuchsin-stained erythrocytes.  $\times 25$ .  
7. A moderately active focus, showing accumulation of various cells and the prominence of vascular spaces. Delafield's hæmatoxylin and eosin.  $\times 125$ .  
8. A group of basic forms apparently recently liberated. In the lower left corner is a half-grown parasite with loose protoplasm.  $\times 1000$ .  
9. Shows an indefinitely outlined phagocyte above and one free in a blood space below. Numerous free parasites are also present. In the lower right corner is a nude body (basic form). The arrow indicates a probable form which resembles a plasma cell.  $\times 1000$ .  
10. A phagocyte containing normal and degenerated parasites. Above is a parasite which has become irregularly outlined, apparently in preparation for subdivision.  $\times 1000$ .  
11. From smear of tissue scrapings. Two endothelial phagocytes containing numerous typical basic parasitic forms. One larger, free parasite also present. Giemsa's stain.  $\times 1000$ .  
12. A similar phagocyte with five basic forms. From the same preparation as fig. 11.

### PLATE III

- FIG. 13. An active focus illustrating the invasion of the epidermis by the infection. Many of the cells that resemble leucocytes are possibly parasites. The condition of the erythrocytes indicates direct connection with the blood channel in the chorium. A granule is budding off of the parasite (?) indicated. Ehrlich's iron hæmatoxylin stain.  $\times 450$ .  
14. Showing several parasites in various stages. A number of round forms, with and without protoplasm, are scattered throughout the preparation. At *a* a large parasite has segmented to produce basic parasitic forms, and at *b* are a number of these forms recently liberated.  $\times 450$ .

## PLATE IV

- FIG. 15. From a Gram-stained smear from a primary culture on ordinary medium. Numerous fine, Gram-positive granules are division forms of the parasite.  $\times 1000$ .
16. Similar to above, from another culture.  $\times 700$ .
17. One appearance of an old, thin, gelatinous form, after Gram's stain.  $\times 400$ .
18. A typical entire-cell form from culture in smeared blood. Giemsa's stain.  $\times 1000$ .
19. Above, an entire-cell form with one refractile spore-granule in nucleus. Below, a later stage, nucleus indistinguishable, the whole containing many such granules. Field slightly out of focus. Giemsa's stain.  $\times 1000$ .
20. Large, soft cell about to liberate group of spores.  $\times 1000$ .
21. Unusually compact mass of spores. On account of plane of focus all but one appear black. Giemsa's stain.  $\times 1000$ .
22. Débris in smear from culture, containing myriads of minute, free spore-granules, stained light blue in the preparation. A number of the larger are clearly discernible. In the center is a darkly stained oval body which corresponds to the basic form. Loeffler's methylene blue.  $\times 825$ .
23. Group of spores beginning to develop. From culture in smeared blood. Giemsa's stain.  $\times 1000$ .
24. Intermediate form containing large metachromatic granules. Developed from spores in smeared blood. Loeffler's methylene blue.  $\times 1000$ .
25. Older protozoönlike cell. Giemsa's stain.  $\times 1000$ .
26. Early stage of gelatinous body, developing from basic form. Giemsa's stain.  $\times 750$ .
27. Intermediate stage of gelatinous body about to differentiate basic forms within itself. Giemsa's stain.  $\times 400$ .
28. Group of basic forms produced within a gelatinous body.  $\times 400$ .

## PLATE V

- FIG. 29. Mass of gelatinous bodies, the protoplasm of which has condensed to form numerous basic forms of various sizes. Somewhat earlier stage than in fig. 27.  $\times 200$ .
30. Large body, from ten-day culture on banana cylinder, containing ordinary round basic forms and bizarre forms of similar material, apparently representing an attempt at mycelial growth.  $\times 500$ .
31. A group of collapsed walls of banana-pulp cells, in smear of material planted on banana cylinder. These might be mistaken for vesicular forms of the gelatinous bodies.  $\times 100$ .
- FIGS. 32 and 33. Empty banana-pulp cells.  $\times 400$ .
- FIG. 34. Segments of aërial hyphæ, from free-growing culture on agar. Tendency to deeper staining at ends. Refractile granules in one segment.  $\times 750$ .
35. Same as above, several cells showing metachromatic granules (black).  $\times 750$ .

## PLATE VI

- FIG. 36. Five-day growth on banana cylinder, illustrating soft, tangled character of aërial growth.

FIGS. 37 and 38. Comparing two-day growth on nutrient banana agar and on banana cylinder.

FIG. 39. Firm "thimble" of growth, seven days on nutrient banana bouillon. Drawn up in tube and turned to demonstrate firmness.

40. Growth on plain bouillon, one week. Cottony aërial growth collapsed except over two small areas.

41. Development of mycelial segments on agar, without oxygen. Early vacuolation.  $\times 450$ .

42. Similar to above. Several very refractile hyalinized segments. Extreme vacuolation of mother cells, which show growth from side.  $\times 450$ .

43. Old mycelium from depths of bouillon. Highly refractile granules formed within the sheath. A delicate septum, to be seen only in occasional senile hyphæ, is indicated.  $\times 450$ .

FIGS. 44 and 45. Two terminal bodies (chlamydospores) found in hanging-drop preparations. That in fig. 45 is not attached to the adjacent filament.  $\times 450$ .







PLATE I.



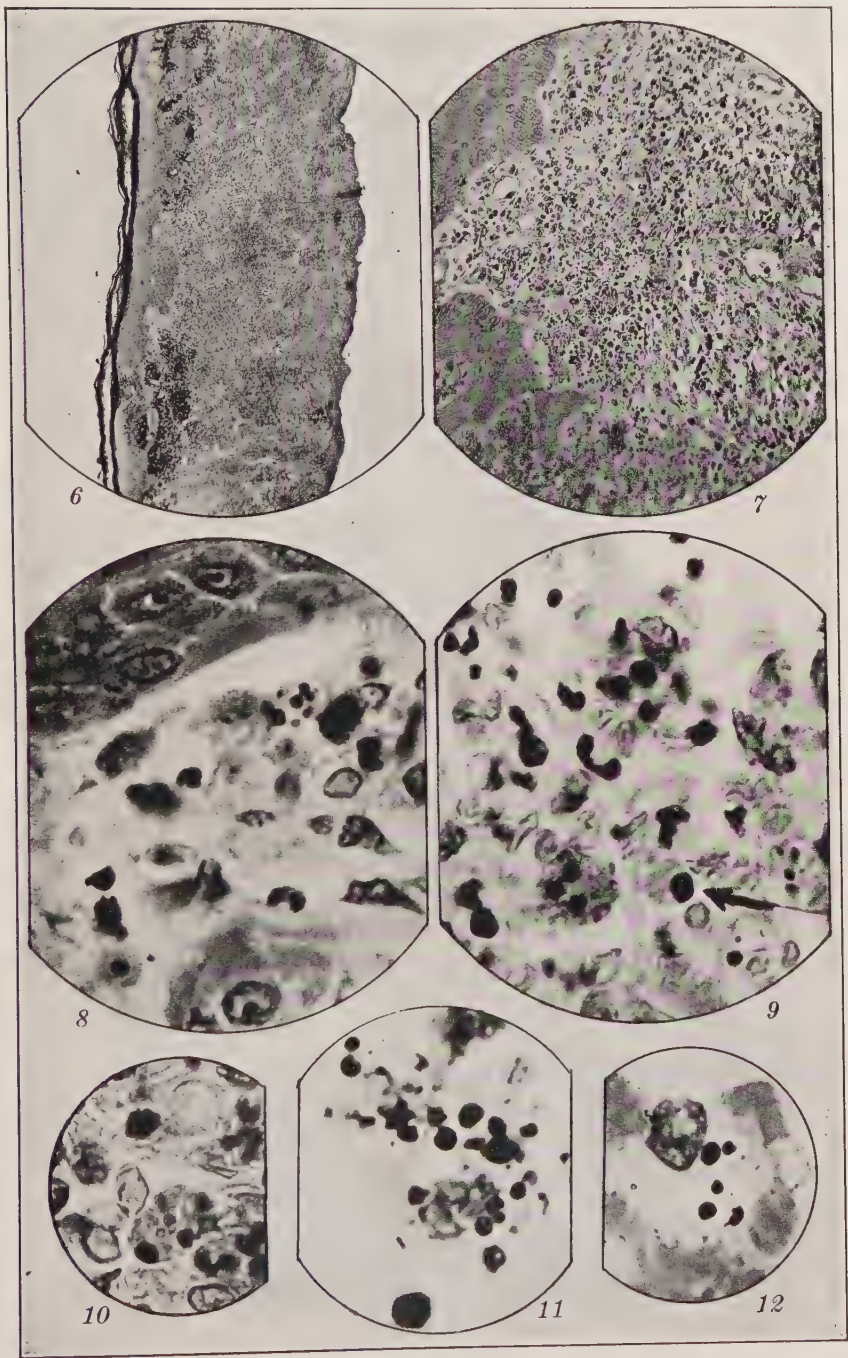


PLATE II.





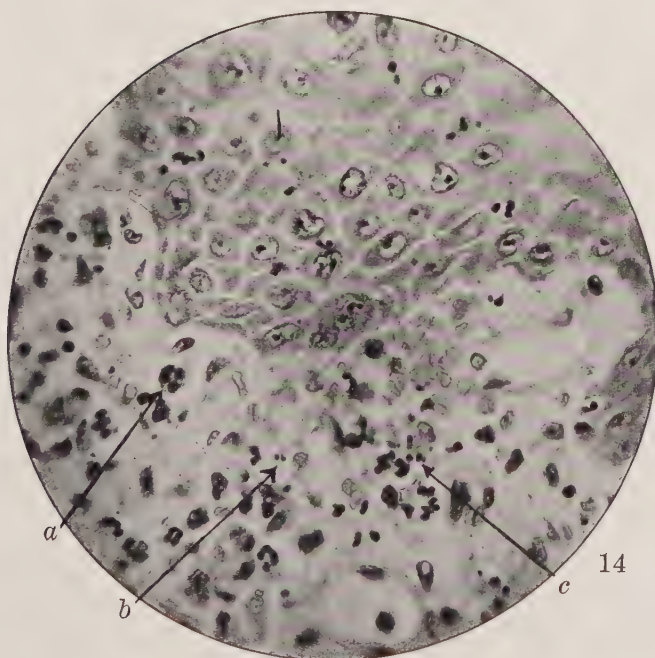
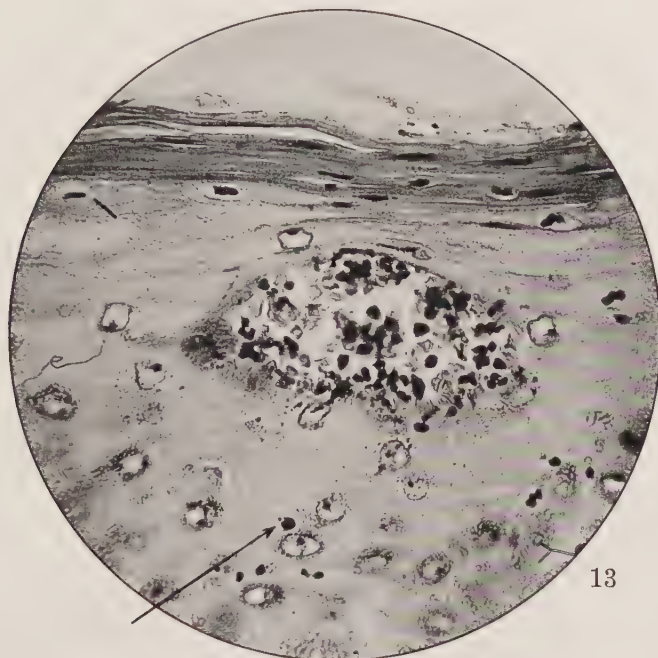


PLATE III.



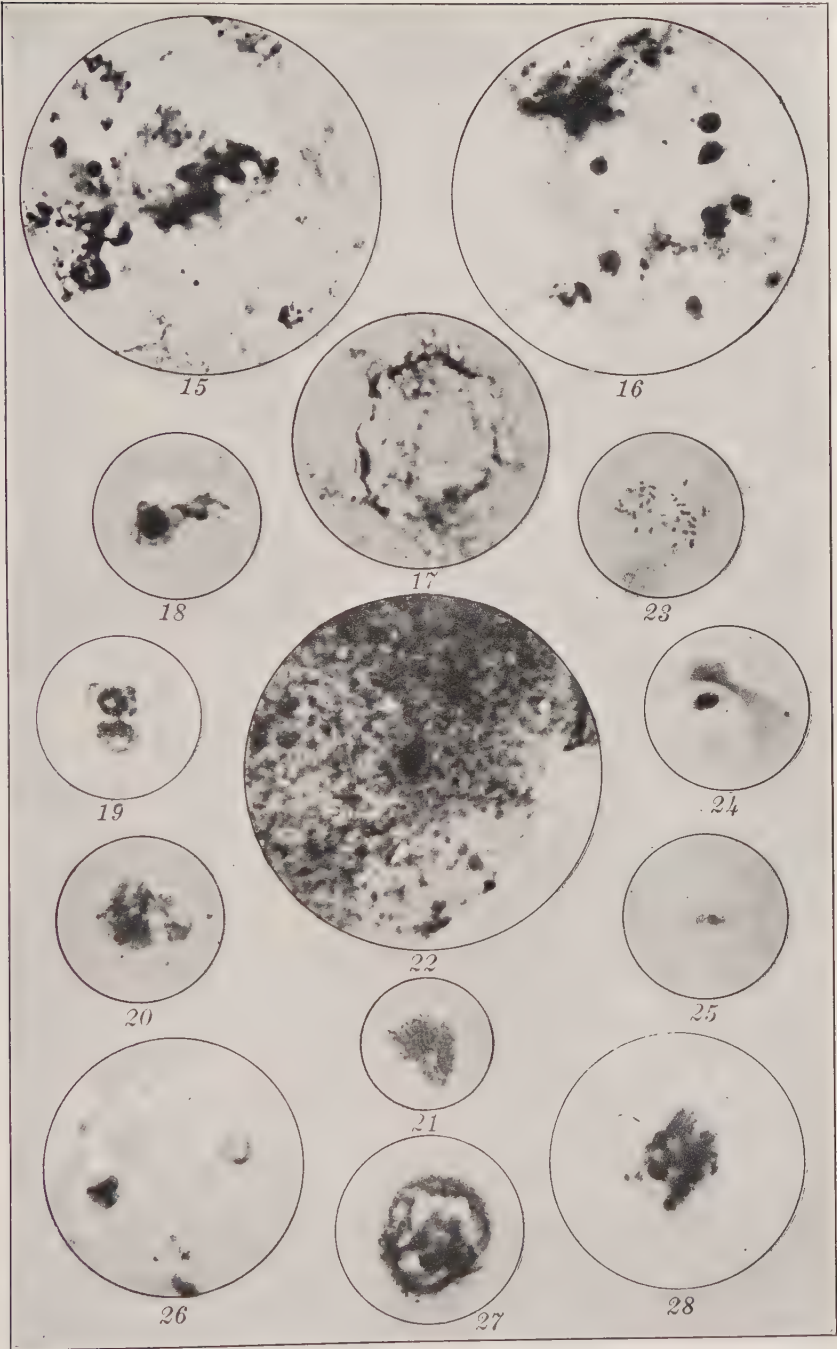


PLATE IV.





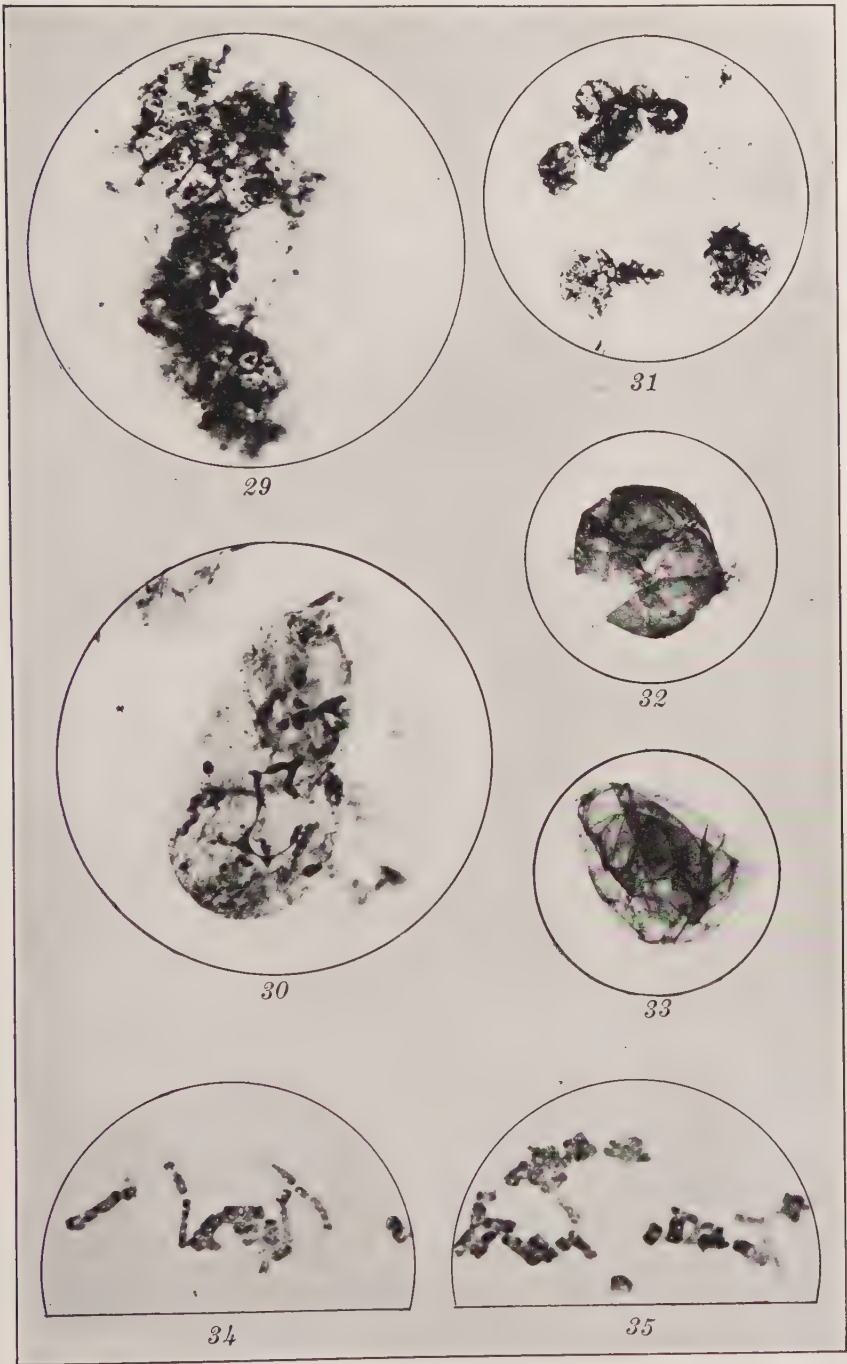


PLATE V.



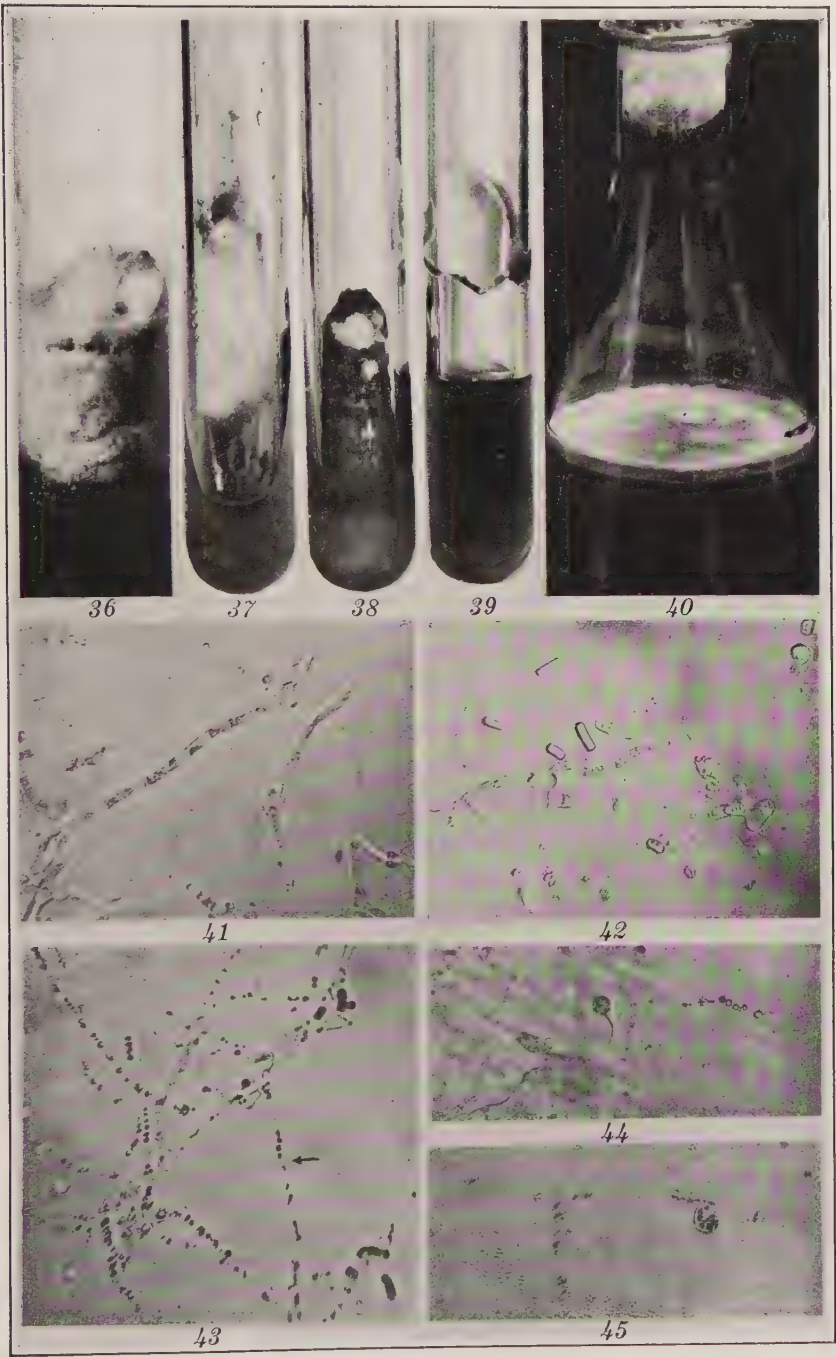


PLATE VI.





## A FATAL PARASITIC INFESTATION IN A HERD OF CATTLE AND GOATS IN AMBOS CAMARINES PROVINCE <sup>1</sup>

By WILLIAM HUTCHINS BOYNTON and LAWRENCE D. WHARTON

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In January, 1916, W. H. Boynton was sent to Ambos Camarines Province to investigate the cause of the death of a large number of animals on a plantation near the town of Minalabag. These animals were kept for breeding purposes in a large pasture, a part of which was high land and a part low rice-paddy land.

The herd was composed of 90 animals immunized by the simultaneous method against rinderpest on March 1, 1915, and of 55 animals that had not been immunized against rinderpest.

The animals that were not immunized were brought from Masbate Island and arrived at the plantation on September 20, 1915. The immunized and the nonimmune animals were kept together.

The owner of the cattle maintained a strict quarantine against outside animals, as there was rinderpest in some of the neighboring districts and he was afraid the nonimmune animals would contract the disease. Every evening the cattle were placed under cover, and smudges were burned around this inclosure during the night to keep off mosquitoes, which are very plentiful in that locality. The animals received an abundance of green feed from the low pasture land. On account of the precautions taken against rinderpest infection, both in having a large number of immunized animals and in quarantining against outside animals, and also on account of the care which the animals received, it was expected that they would thrive.

Practically all the animals were in good condition when they arrived at the plantation. After a time many of them began to lose weight, even with the feed and care which they received, and as the caretaker states, they became thinner and thinner and finally died, although they ate well until just before death.

A considerable number died during and just after the heavy storms, which indicated that they did not have vitality enough to withstand these storms.

There were also 60 head of goats on the place, and at the time of Boynton's visit all but 11 had died and one of

<sup>1</sup> Reprinted from *Phil. Agr. Rev.* (1916), 9, 348.

these was sick. The sick goat was emaciated, was very anæmic in appearance, stood in the sun with his back arched and head down, had a slight diarrhœa, and possessed very little life, moving with difficulty when urged. The caretaker stated that this was the general appearance of the greater percentage of those that had died.

This animal was killed and autopsied. The blood was very anæmic in appearance. There was practically no fat in the peritoneal cavity, the organs were pale, the mesenteric lymphatics were somewhat enlarged and soft, and upon section a watery serous fluid oozed from them. The fourth stomach and the duodenum contained myriads of worms—red from imbibing the animal's blood—and small petechiæ were scattered over the mucous membrane of these organs. There were many worms of a different character in the cæcum and in the colon. The enormous number of these intestinal parasites was undoubtedly accountable for the condition of this animal. Specimens of these worms were preserved and later were identified by L. D. Wharton.

There had been no deaths among the cattle for some time prior to Boynton's visit, but two animals were very thin, and the caretaker stated that they had exactly the same appearance as those that had died. For this reason one was killed and autopsied to see if the cause of the condition could be ascertained.

Upon autopsy the internal organs were found to be anæmic; the heart, the kidneys, the spleen, and the liver were normal, except for their pale appearance. Worms were found in the bronchioles of the lungs, and there was a slight pneumonia in the left principal lobe. Many worms were found in the fourth stomach, the small intestines, the cæcum, and the upper part of the colon. There was a marked catarrhal enteritis in the ileum and numerous petechiæ. In many instances the worms were dislodged from the mucosa with some difficulty. Specimens of the worms found in the various parts were preserved and later were identified by Wharton.

The large number of these intestinal parasites was undoubtedly the direct cause of the emaciated and anæmic condition of this animal.

From the autopsies of the two animals picked out by the caretaker as being identical in appearance with many of the animals that had died, it is probable that a majority of the latter were also heavily infested with intestinal parasites and that these parasites were the direct cause of death.

It will be noted from Table I that 11 cattle died during the first storm on October 23 and that 14 died during the second storm on November 3. This indicates that their vitality was so low that the animals could not withstand exposure when infested to such an extent with these intestinal parasites.

The caretaker was directed to transfer the entire herd to grazing lands on high ground, and this was done. On April 15, 1916, the Bureau of Agriculture was notified that only two of the cattle had died after being transferred to the high ground, and that the whole herd was in good condition with the exception of one animal which had not completely recovered. This fact demonstrated that the animals had become infested on the low-lying grazing grounds.

TABLE I.—Showing the number of deaths from intestinal parasites in a herd of cattle and goats in Ambos Camarines Province. Total deaths, 69.

Date.	Died.		Date.	Died.	
	Immunized.	Non-immunized. <sup>a</sup>		Immunized.	Non-immunized. <sup>a</sup>
1915.			1915.		
Mar. 6 .....	1		Oct. 15 .....		1
Mar. 28 .....	1		Oct. 20 .....		1
Apr. 17 .....	1		Oct. 22 .....		1
May 9 .....	1		Oct. 23 <sup>b</sup> .....	5	6
June 2 .....	3		Nov. 3 <sup>c</sup> .....	10	4
June 3 .....	1		Dec. 3 .....	1	
June 9 .....	1		Dec. 4 .....		1
Aug. 1-31 .....	9		Dec. 7 .....	2	1
Sept. 20 .....		1	Dec. 8 .....	1	
Sept. 25 .....	2	2	Dec. 9 .....		1
Sept. 28 .....	2		Dec. 22 .....		1
Oct. 7 .....		2	Dec. 23 <sup>d</sup> .....	2	
Oct. 12 .....		1			
Oct. 13 .....		3	Total .....	43	26

<sup>a</sup> The nonimmunized animals arrived from Masbate on September 20, 1915.

<sup>b</sup> First typhoon.

<sup>c</sup> Second typhoon.

<sup>d</sup> Third typhoon.

The collection of worms made by Boynton was found to contain 8 species, 7 from the cow and 2 from the goat. One species, *Haemonchus contortus*, was found in both individuals. The species, as far as they can be identified with the limited literature at hand, are as follows:

## NEMATODES

## STRONGYLIDÆ

*Bunostomum phlebotomum* (Railliet, 1902).

*Strongylus radiatus*, *Dochmius radiatus*, *Uncinaria radiata*, *Monodontus phlebotomus*.

These are small white worms with a large, armed buccal capsule, bent dorsally. There is a short, dorsal buccal tooth; a pair of ventral buccal teeth; and a pair of small, subventral lancets; all attached to the base of the capsule. There are six circumoral papillæ and a pair of short, blunt cervical papillæ opposite the anterior portion of the œsophagus. The œsophagus is enlarged at the posterior end.

The males are 10 to 14 millimeters long and about 0.5 millimeter in thickness. The dorsal lobe of the bursa copulatrix is shorter than the lateral lobes. The dorsal ray is asymmetrical, coming off from the base of the left lateral ray and giving off the externolaterals asymmetrically. The spicules are long and filiform.

The females are 15 to 20 millimeters in length and a little thicker than the males. The posterior end of the body is attenuated and terminates in a slender, bluntly pointed tail behind the anus. The vulva is situated a little in front of the middle of the body.

The above were collected from the ileum and the fourth stomach of a cow (*Bos taurus*) at Minalabag, Camarines, and are listed as specimen No. 110, helminthological collection, College of Medicine and Surgery.

*Oesophagostomum columbianum* (Curtice, 1890).

This is the common *Oesophagostomum* of sheep and goats. It undergoes its development in nodules in the walls of the intestines and leaves these nodules only when it has reached the adult stage, at which time it passes into the lumen of the intestine, where copulation and egg-laying occur.

The mouth is surrounded by a slightly inflated mouth collar. There are well-developed lateral membranes extending posteriorly from the transverse groove along the whole length of the body. A pair of cervical papillæ are present in front of the middle of the œsophagus.

The males are 10 to 16 millimeters long and have a bursa. The females are 14 to 20 millimeters long with the vulva close to the end of the tail.

The above were collected from the intestine of a goat at Mina-



labag, Camarines, and are listed as specimen No. 106, helminthological collection, College of Medicine and Surgery.

*Oesophagostomum* sp.

Worms of this genus were also collected from the cow. While they more closely resemble *O. radiatum* than any other described form, it is believed that they should be placed in a separate species. The mouth collar is more prominent than in *O. radiatum*, the cervical inflation of the cuticle is strongly pronounced, and the body is slightly larger than that of *O. radiatum*. The externodorsal rays of the bursa of the males originate very close to the root of the dorsal ray; and the external divisions of the dorsal ray are knob-shaped, while the internal divisions are very long and slender.

The above were collected from the cæcum of a cow, at Minalabag, Camarines, and are listed as specimen No. 104, helminthological collection, College of Medicine and Surgery.

#### TRICHOSTRONGYLIDÆ

*Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898.

*Strongylus contortus*.

This is the common "wire worm" of cattle, sheep, and goats. The head is small and bears a minute buccal lancet. There are two cervical spines about 0.3 to 0.4 millimeter from the anterior end of the body. The œsophagus is claviform. The body is long and slender and is attenuated toward both ends.

These worms were found in the fourth stomach of both the goat and the cow at Minalabag, Camarines, and are listed as specimens Nos. 102 and 108, helminthological collection, College of Medicine and Surgery.

*Nematode* sp.—Several female worms, 30 to 35 millimeters in length and having a diameter of about 0.6 millimeter, were collected from the fourth stomach of the cow. They probably belong to the family Trichostrongylidæ, although they could not be identified further. They are filled with large thin-shelled eggs averaging 107 microns in length by 44 microns in diameter. The vulva is situated about 1 millimeter in front of the anus, and the tail is short and is sharply pointed behind the anus. They are listed as specimen No. 115, helminthological collection, College of Medicine and Surgery.

#### TRICHINELLIDÆ

*Trichuris ovis* (Abildgaard, 1795) Smith, 1908.

*Trichocephalus affinis*.

This is the common whipworm of cattle and sheep. Specimens

measure from 45 to 70 millimeters in length, the anterior slender portion of the body making up from two thirds to three fourths of the whole length.

The posterior end of the male is curved spirally and bears a single spicule surrounded by a spiny sheath with a bulbous enlargement at the end. The posterior end of the female is straight and is filled with lemon-shaped eggs.

These were collected from the cæcum of a cow at Minalabag, Camarines, and are listed as specimen No. 103, helminthological collection, College of Medicine and Surgery.

#### FILARIIDÆ

*Filaria labiato-papillosa*.

These are long, slender, threadlike white worms with a terminal mouth surrounded by a chitinous ring bearing two papillalike processes.

The males are 4 centimeters long with a spiral, pointed tail, bearing four pairs of preanal and five postanal papillæ and two unequal spicules.

The females are 6 to 7 centimeters long. The posterior end is slightly curved and terminates in a fasciculus of small blunt points preceded by two thick, cortical, lateral papillæ.

These worms were found free in the abdominal cavity and in the lungs of a cow, Minalabag, Camarines, and are listed as specimen No. 114, helminthological collection, College of Medicine and Surgery.

#### TREMATODES

*Paramphistomum* sp.

A single small specimen of this genus was found in the stomach of the cow.

It is immature, and so the species could not be determined. It is listed as specimen No. 112, helminthological collection, College of Medicine and Surgery.

## REVIEWS

The | Non-surgical | Treatment | of | Intestinal Stasis | and Constipation |  
compiled by | Robert H. Ferguson, M. D., Sc. D. | also an important  
announcement | regarding liquid petrolatum | published for the phy-  
sician and surgeon by | E. R. Squibb & Sons, New York | Medical  
Department | MCMXVI | Cloth, pp. 1-109.

The title of this little volume is misleading. A few pages are devoted to the definition of intestinal stasis, the cause of it, its importance, and its remote effects. Most of the volume is a collection of favorable statements concerning liquid petrolatum as a remedy for intestinal stasis. The statements are excerpts from published articles which may or may not include records of experiments, but with the exception of the mention of four cases, the book itself offers no experimental data.

J. L. BOOTH.

Diseases | of the | Digestive Tract | and | Their Treatment | by | A. Everett  
Austin, A. M., M. D. | [11 lines] | St. Louis | C. V. Mosby Company |  
1916 | Cloth, pp. 1-552. Price \$5.50.

A striking feature of this pretentious volume is the very general lack of proportion. Nearly one third of it is taken up with the various methods of examination and diagnosis, with special emphasis on the X-ray. The physiology of digestion and the chemistry of the digestive tract, both in health and disease, are elaborately detailed. On the other hand, barring a few words on the examination of the mouth, teeth, and pharynx, the part of the tract above the stomach is completely ignored, while the diseases of the tonsils and pyorrhœa alveolaris are not mentioned.

As a contrast, seven pages and six illustrations are given to the stomach tube and its administration, while one third of a page suffices for *Entamœba coli* and *E. histolytica* and the havoc wrought by them. No other protozoa are mentioned. The Shiga-Flexner group of dysentery bacilli are dismissed with a three-line notice. Typhoid enteritis is not discussed.

Intestinal parasites, the diseases caused by them, and their treatment occupy only six pages, of which one is considered sufficient for hookworm infection.

The illustrations of *Oxyuris vermicularis* and *Ankylostoma duodenale*, marked natural size, are two and one-half times too large and do not remotely resemble these worms.

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Local and Regional | Anesthesia | [4 lines] | by | Carroll W. Allen, M. D. |  
 [3 lines] | with an introduction by | Rudolph Matas, M. D. | [2 lines] |  
 illustrated | Philadelphia and London | W. B. Saunders Company |  
 1915 | Pages 1-625. Cloth, \$6 net; half morocco, \$7.50 net.

Allen's Local and Regional Anesthesia contains a most extensive work on local anesthesia. This branch of modern surgery has assumed a foremost importance of late years, and Dr. Allen's work is a most valuable contribution to the science. The description and technic is easy and comprehensive and well repays study.

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Surgery | of the | Blood Vessels | by | J. Shelton Horsley, M. D., F. A. C.  
 S. | [5 lines] | illustrated | St. Louis | C. V. Mosby Company | 1915 |  
 Cloth, pp. 1-304. Price \$4.

Horsley's Surgery of the Blood Vessels contains some of the latest work on this subject and opens a fresh field for further extensive research. The style is fluent, comprehensive, and interesting. The book will be most useful to the modern surgeon.

A. VAZQUEZ.



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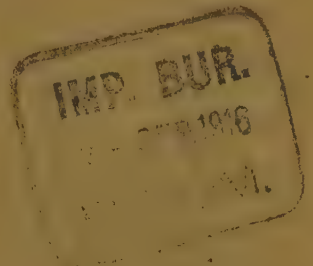
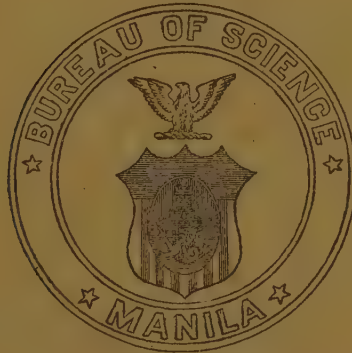
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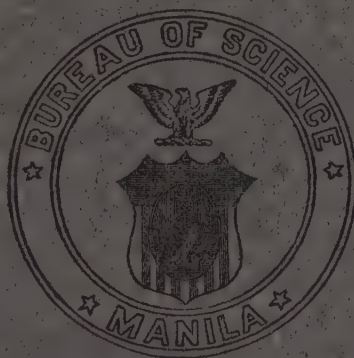
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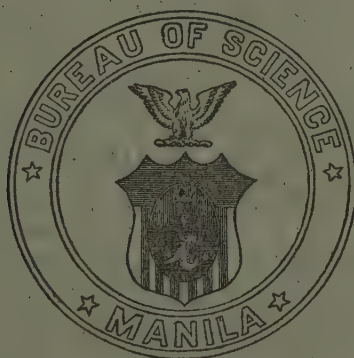
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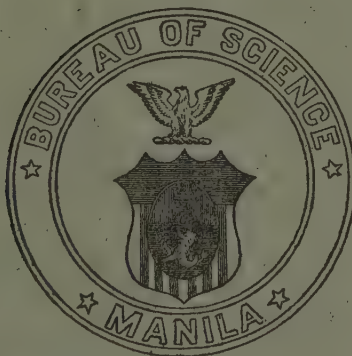
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